VOL. XII, No. 2 MARCH 1960

UNIVERSITY OF HAWAII
LIBRARY
MAR 17 '60

PLANT AND SOIL

International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

MARTINUS NIJHOFF — PUBLISHER — THE HAGUE

PLANT AND SOIL

International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

Revue internationale de nutrition des plantes, de chimie végétale, de microbiologie du sol et des maladies des plantes en rapport avec le sol

Internationale Zeitschrift für Pflanzenernährung, Pflanzenchemie, Bodenmikrobiologie und bodenbedingte Pflanzenkrankheiten

Issued under the auspices of the "Koninklijk Genootschap voor Landbouwwetenschap" (Royal Netherlands Society of Agricultural Science)

Executive Editors: D. I. Arnon, Berkeley; J. Baeyens, Louvain; F. C. Gerretsen, Groningen; G. W. Harmsen, Groningen; E. G. Mulder, Wageningen; F. J. Richards, London; A. C. Schuffelen, Wageningen; F. Steenbjerg, Copenhagen. **Secretary:** G. H. Arnold, Groningen.

Consulting Editors: Wm. A. Albrecht, Columbia, Mo.; C. Arnaudi, Milan; E. Gäumann, Zurich; L. Gisiger, Liebefeld-Berne; F. G. Gregory, London; A. J. de Groot, Groningen; W. R. C. Handley, Oxford; J. L. Harley, Oxford; J. den Holder, Ghent; V. J. Koningsberger, Utrecht; J. Lavollay, Paris; L. Leyton, Oxford; W. J. Lütjeharms, Bloemfontein; H. Lundegårdh, Uppsala; E. Melin, Uppsala; M. Ødelien, Ås; E. J. Petersen, Copenhagen; C. S. Piper, Adelaide; D. Snow, Reading; H. G. Thornton, Harpenden, Herts.; S. Tovborg Jensen, Copenhagen; A. I. Virtanen, Helsinki; S. A. Waksman, New Brunswick; F. W. Went, Pasadena; Joh^{a}. Westerdijk, Baarn; L. K. Wiersum, Groningen; J. G. Wood, Adelaide.

CONTENTS: TABLE DES MATIÈRES: INHALT:

E. A. N. Greenwood and *E. G. Hallsworth*, Studies on the nutrition of forage legumes. II. Some interactions of calcium, phosphorus, copper and molybdenum on the growth and chemical composition of *Trifolium subterraneum* L. 97

P. C. DeKock, A. Hall and *Margaret McDonald*, A relation between the ratios of phosphorus to iron and potassium to calcium in mustard leaves. 128

K. C. Marshall and *M. Alexander*, Competition between soil bacteria and fusarium. 143

D. J. Greenland and *J. M. L. Kowal*, Nutrient content of the moist tropical forest of Ghana 154

D. J. Greenwood and *H. Lees*, Studies on the decomposition of amino acids in soils. III. The process of amino acid aerobic decomposition and some properties of amino acid-oxidizing organisms. 175

Letter to the Editors, *D. Claus* und *Kl. Mechsner*, Ueber die Brauchbarkeit der von Ed. Hofmann ausgearbeiteten Methoden zur Bestimmung der Enzyme im Boden 195

Printed in the Netherlands

Plant and Soil XII, 97-198 The Hague March 1960

Plant and Soil XII, no 2 March 1960

STUDIES ON THE NUTRITION OF FORAGE LEGUMES

II. SOME INTERACTIONS OF CALCIUM, PHOSPHORUS, COPPER AND MOLYBDENUM ON THE GROWTH AND CHEMICAL COMPOSITION OF *TRIFOLIUM SUBTERRANEUM* L.

by E. A. N. GREENWOOD and E. G. HALLSWORTH

Department of Agricultural Sciences
University of Nottingham School of Agriculture

INTRODUCTION

The object of the work described in this paper was to study some interactions between nutrients whose separate effects on temperate legumes have long been demonstrated, and which have been considered to influence nodulation. From this it was hoped that a more comprehensive understanding might be obtained as to the nutrient requirement of symbiotic plants.

The test plant, subterranean clover, was used because it is an excellent experimental plant. Its radial, prostrate habit requires no aerial support, it does not form adventitious roots and it withstands very high glasshouse temperatures without wilting. Furthermore, a measurement of its radius, which is highly correlated with dry-matter yield in its vegetative stage can be rapidly performed and it was considered that this would help to keep track of the replicates widely dispersed by randomization in large factorial experiments.

The mineral nutrition of subterranean clover has received much attention. Its responses to the essential elements are well known and deficiency symptoms have been comprehensively described and illustrated by Millikan [10] and others. However, the yield response of a plant to the supply of a given element may depend on the levels of other nutrients available, and many interactions of one nutrient on another have been reported. It is not always possible from the information given to propose a mechanism for these,

since the interaction may simply be an indirect effect of altered growth rate. In the case of legumes growing normally in the field, where a large part of their nitrogen supply may be derived from symbiosis with *Rhizobium spp.*, there is the added possibility that growth rate may also be influenced through the effects of various levels of nutrients on nitrogen fixation. In sand- or water-culture experiments such effects may not be apparent if sufficient combined nitrogen is available in the nutrient solution.

In addition to the yield, the concentrations and absolute quantities of other elements may be changed by interactions between nutrient variables and these alterations may affect the growth of plants or the health of animals grazing them.

For convenience in this paper, the term *indirect* effect of a nutrient variable on chemical composition is defined as one which has been caused by a differential growth rate due to treatments. A *direct* effect is one which is produced by a variable independently of any effect on growth rate.

In the work described here the controlled variables are the supply of calcium, copper, phosphorus, and molybdenum, combined nitrogen being supplied as sodium nitrate, either at a very high rate in order virtually to eliminate nitrogen fixation, or at a very low rate to allow symbiosis to become the principal source of nitrogen. An attempt is made to segregate the *direct* from the *indirect* effects of interaction on the chemical composition of the plants.

EXPERIMENTAL METHODS

In general, the sand-culture technique of Hewitt [9] was adopted. Bedford silversand, steam-washed with 1 : 1 HCl was contained in seven-inch 'Bituros' * painted pots, with the centre drainage hole covered with a watch glass. The water capacity of the sand was about 700 ml per pot. Solutions were renewed by flushing with an excess volume every three days.

The nutrient solution used is given in Table 1 and, the chronological data are presented in Table 2. It is to be noted that of the three experiments reported here, B was a springtime repetition of A, and that B and C were concurrent and collateral and had several factorial levels in common.

Since none of the levels of variables was critically low from the point of view of contamination, chemicals of 'A.R.' quality only were used. Tap water was run through a portable Permutit ion exchanger containing separate beds of De-acidite E and Zeocarb H.I. This provided water of conductivity 4–6 micromho.

* 'Bituros' manufactured by Wales Dove Bitumastic Ltd.

p. 99. Table 1. Concentration of Fe "84" should read "4.2"

Concentrations (ppm) and source of the nutrient solutions used				
Variable components				
Element	Experiment			Source
	A	B	C	
N	224	12	224	$NaNO_3$
Ca	64	64	64	$CaCl_2.6H_2O$
	8	8	16	
			8	
P	80	80	40	$NaH_2PO_4.2H_2O$
	40	40	8	
	8	8	4	
Cu	0.064	0.064	0.064	$CuSO_4.5H_2O$
	0.0064	0.0064	0.0064	
Mo	0.40	0.40	0.40	$(NH_4)_6.Mo_7O_{24}.4H_2O$
	0.04	0.04	0.004	
	0.004	0.004		
Constant components				
Element	All experiments			Source
K	78			KCl
Mg	18			$MgSO_4$
S	24 (minimum)			$MgSO_4$
Fe	84			Ferric citrate
Mn	0.55			$MnSO_4.4H_2O$
Zn	0.065			$ZnSO_4.7H_2O$
B	0.37			H_3BO_3
Al (Expt. A only)	1.8			$Al_2(SO_4)_3.18H_2O$

The initial pH of the solution was 4.5 $\pm$ 0.1, depending on the levels of variables. In Experiment A, where aluminium sulphate was added, it fell to pH 4.2. The control was not good, as might be expected with sodium nitrate (Hewitt [9]).

Seeds of subterranean clover – *Trifolium subterraneum* L – strain Mt.

TABLE 2

Dates of experiments				
Experiment	Season	Germination	Harvest	Age in weeks
A.1	Summer/autumn	20.7.1955	30. 9.1955	10
2	Summer/autumn	2.8.1955	19.11.1955	15½
B	Spring/summer	22.3.1956	26. 6.1956	13½
C	Spring/summer	22.3.1956	26. 6.1956	13½

Barker *, were germinated on filter paper in distilled water. Seeds intended for symbiotic experiments were inoculated at radicle emergence with a suspension of *Rhizobium subterraneum*. At this stage all seeds were broadcast into unwashed Bedford sand until development of the first trifoliate leaf, when a selection for uniformity was made, based on top and root development. Selected seedlings were transplanted separately into pots of washed sand.

Each treatment was replicated seven times (eight for 1st. harvest Experiment A) and the pots randomised across the glasshouse.

Chemical analysis

Plant material from Experiments A and B were hand ground, but the large stemmy plants of Experiment C were ground in a "Christie-Norris" mill in order to obtain a uniform sample.

Two methods of digestion were used:

(1) Sulphuric acid with sodium sulphate and selenium. This was carried out using 10 ml H_2SO_4 in a Kjeldahl digestion flask until completely colourless, diluted to 200 ml and aliquots taken for the determination of N, P and Ca.

(2) Nitric acid and perchloric acid followed by hydrogen peroxide. The digest was taken up in 10 ml HCl, diluted to 100 ml and aliquots taken for Cu and Mo determinations.

Nitrogen was determined by macro Kjeldahl; phosphorus by reduction of phosphomolybdate with stannous chloride; copper by carbamate and extraction with amyl alcohol; molybdenum by the thiocyanate–stannous chloride method of Purvis and Peterson [13] and extraction with amyl alcohol; calcium by adding excess EDTA, and allowing 15 minutes before backtitrating with calcium against the fluorescent indicator "calcein" (Hopkins and Williams).

Growth measurement

At weekly intervals, measurement of the maximum radius of the clover rosette was recorded to follow more closely the changes in dry weight. The validity of this measurement as an index of dry weight in the vegetative stage of growth is discussed elsewhere (Greenwood and Hallsworth [7]).

OVERALL EFFECTS OF VARIABLES ON YIELD, SYMPTOMS AND NODULATION

In order to simplify notation, levels of controlled variables are hereafter referred to as the chemical symbol followed by the concentration value in ppm. Thus, for example, 12 ppm N becomes N12.

Data are presented in logarithmic and arithmetic form in Table 3. Analyses of variance for yield data were performed on the former because it was more suitable. Calcium showed the greatest change in variance of arithmetic

* Supplied by Arthur Yates & Sons, Pty. Ltd., Sydney, Australia.

values and this was corrected by transformation. Other variables, however, were not improved by transformation but the differences were not so serious.

Levels of significance in most tables are shown by tailed arrows pointing always from a lower to a higher value. The number of tails corresponds with the conventional use of asterisks thus: ⟶ (10%); ⟶ (5%); ⟶ (1%); ⟶ (0.1%).

TABLE 3

Overall effect of controlled variables on dry-matter yield of the whole plant given as log 10x. Values in parenthesis refer to dry weight in g

Expt.	P80	P40	P8	P4	$P =$ 0.05	$P =$ 0.01
A.1	1.13 (1.49)	1.19 (1.75)	1.18 (1.73)	—	0.04	0.05
A.2	1.81 (7.8)	1.81 (8.2)	1.77 (7.1)	—	0.05	0.07
B (N12)	1.27 (1.20)	1.28 (2.10)	1.49 (3.91)	—	0.04	0.06
C (N224)	—	1.81 (8.0)	1.87 (9.0)	1.71 (6.2)	0.05	0.07
	Ca64	Ca16	Ca8			
A.1	1.28 (2.07)	—	1.06 (1.25)		0.03	0.04
A.2	1.97 (10.8)	—	1.62 (4.5)		0.04	0.05
B (N12)	1.48 (3.63)	—	1.21 (1.71)		0.04	0.05
C (N224)	2.01 (11.3)	1.83 (7.7)	1.54 (4.2)		0.05	0.07
	Mo 0.40	Mo 0.04	Mo 0.004			
A.1	1.17 (1.66)	1.18 (1.69)	1.16 (1.63)		0.04	0.05
A.2	1.85 (8.2)	1.78 (7.6)	1.76 (7.3)		0.05	0.07
B (N12)	1.37 (2.81)	1.32 (2.45)	1.35 (2.77)		0.04	0.06
C (N224)	1.78 (7.6)	—	1.81 (7.9)		0.04	0.06
	Cu 0.064		Cu 0.0064			
A.1	1.28 (2.06)		1.06 (1.27)		0.03	0.04
A.2	1.92 (10.0)		1.67 (5.4)		0.04	0.05
B (N12)	1.40 (3.21)		1.29 (2.13)		0.04	0.05
C (N224)	1.95 (10.1)		1.65 (5.4)		0.04	0.06

Nitrogen

N12 produced smaller plants than N224, with much less dry matter, but allowed for the possible development of an active symbiotic system. The extent to which this occurred depended on the suitability of the levels of other variables for symbiosis. Where the levels of nitrate N were very high *i.e.* Experiments A and C, there was no visible evidence of effective nodulation. In Experiment A, nodules were absent, and in C, they were greenish brown, minute, and numerous. In Experiment B, under favourable nutrient treatments, nodules were pinkish white, few, very large, complex, and situated on the upper part of the primary roots. A few smaller, simple nodules were on the distal, young roots.

The growth habit of the plants, as well as the time of inflorescence, was affected by the nitrogen level. Plate 1 illustrates the prostrate habit on N12 and the more erect growth on N224. Inflorescence appeared two weeks earlier on low N. The mean dry weight of the N12 plants was 8.2 g, and of the N224 plants, 16.4 g.

Calcium

The overall yield differences due to calcium levels were very large (Table 3), and severe deficiency symptoms occurred on the Ca16 and Ca8 treatments.

On a high-nitrate regime the first clinical symptom was a change in leaf colour from bright to dull green, quickly followed by disappearance of the brown anthocyanin markings of leaflets. New leaves emerged dwarfed and pinched with necrotic tips. Petioles of older leaves collapsed, with necrosis working back toward the base. Leaflets attached to such petioles remained fresh and green for some time, but showed a characteristic "water-soak" spot on the underside, but eventually these also died. Leaflets of intact petioles, however, became severely necrotic at the apical margins and finally total necrosis developed. Roots were also necrotic. These symptoms were similar to those described by Millikan [10].

In the symbiotic system (Experiment B) the effect of calcium deficiency was a general chlorosis due to inhibited nitrogen fixation. The effect of a low calcium supply on symbiosis was also seen in the appearance of nodules. These were smaller, greenish brown and sometimes necrotic, but at Ca64, nodules were large and apparently healthy, providing other variate levels were suitable.

The intensity of calcium-deficiency symptoms at N224 was noticeably influenced by solar radiation (recorded by a Kipp solarimeter situated inside the house). Fig. 1 shows this, using maximum radius as the index of the dry weight of non-necrotic tissue. The solarimeter histogram shows that radiation increased to a maximum after the eighth week, a few days after which the index commenced to fall for Ca8 until about one week after the arrival of dull weather (week 11) when a rapid, partial recovery was made.

Under both nitrogen regimes, the high phosphorus levels caused an earlier onset and greater severity of calcium-deficiency symptoms. This interaction is described later.

Plate 1. Effect of nitrogen supply on growth habit.
(Expts. B & C)
Left : 224 ppmN. Right : 12 ppmN.

Plate 2. Effect of phosphorus levels on roots and nodules (Exp. B)
Left : 8 ppmP. Right : 80 ppmP.

Plate 3. Effect of phosphorus on calcium deficiency (Exp. C)
Left: 8 ppm P Right: 40 ppm P

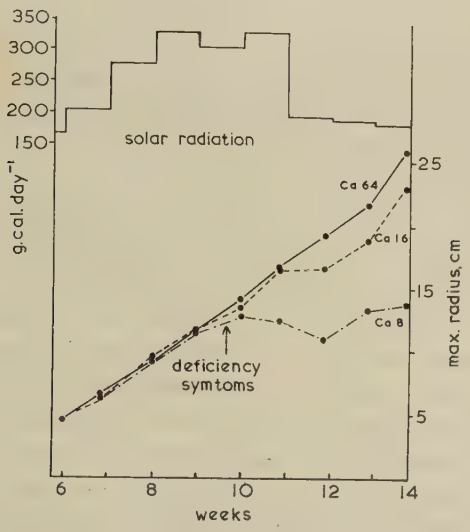


Fig. 1. Relation between solar radiation (under glass) and calcium deficiency (Exp. C)

Phosphorus

The phosphorus nutrient level was based on that of Long Ashton (41 ppm). In Experiment A, second harvest, the high phosphorus level, P80, gave no increase in yield over P40, and the level P8 allowed yields almost as great, and in some treatments, greater than those for P40 and P80. Thus the overall yields of plants on treatments P80–40–8 in this experiment were not significantly different (Table 3). Under the same high-nitrogen regime and consequent rapid growth in the following spring (Experiment C) this effect was repeated for the levels P40 and P8. The lowest phosphorus treatment, P4, was insufficient for optimum growth as shown by a highly significant depression of yield. These plants were, however, without any symptoms of phosphorus deficiency.

In the case of symbiotic plants of Experiment B (N12), the depressing effect of the higher phosphorus levels on yield was significant and large. Further, P80 and P40 plants were seriously necrotic. This necrosis involved first the distal end of the leaflet, then the entire blade and finally the petiole. Plants suffered most acutely during periods of high insolation. Concurrently, P8 plants increased in vigour and appeared normal. The effect of phosphorus level on the growth of roots and nodules is illustrated in Plate 2.

Copper

The higher of the copper levels was that of Long Ashton nutrient solution (0.064 ppm) and the lower was one-tenth of this. The yield differences between these two levels were large and occurred to varying extents under nearly all treatments on symbiotic and non-symbiotic plants and have been clearly repeated several times in different seasons. The effect is presented in Table 3.

Low-copper plants on the high-nitrogen regime in many respects appeared healthy, but although there were no lesions, they showed characteristic morphology and colour. For several weeks they appeared as small replicas of Cu 0.064 plants. Later the petioles became long and spindley and leaf numbers fell. Thus in Experiment A, 1st harvest, at the age of 10 weeks, leaf numbers per plant on the highest calcium level were 64 for high copper and 48 for low copper (means of 72 plants). With the occurrence of high insolation, red pigmentation of the leaflet developed and became intense, but petiole and runner remained green. Once established, the intensity of the red varied according to the fluctuations in solar energy. Under symbiotic conditions, low-copper plants also showed intense red pigmentation, together with lower yields and mild general chlorosis suggesting nitrogen deficiency.

Molybdenum

All molybdenum levels used were high, and the yield responses were consequently small (Table 3). Interactions of molybdenum with other variables have occurred, however, and these will be reported later. Molybdenum treatments, whether accompanied by yield effects or not, produced no clinical symptoms, and nodulation seemed independent of these three levels but no measurement of this was undertaken.

SIGNIFICANT INTERACTIONS OF VARIABLES ON YIELD

Whenever overall yield effects of controlled variables are large it might be expected that these will require qualification by their interactions. Table 4 lists these for each experiment together with their respective variance ratios to give an indication of their magnitude.

TABLE 4

Significant interactions on yield (log transformation)				
Interaction	Variance ratio			
	Experiment A		Experiment B	Experiment C
	1st harvest, tops	2nd harvest, whole plant	Whole plant	Whole plant
Ca × Cu	—	36	14.9	—
Ca × Mo	—	7.9	—	—
Ca × P	—	11.0	14.8	8.3
Cu × P	5.8	6.4	35	5.6
Ca × Cu × Mo	—	4.0	—	—

Interaction of calcium and copper

This occurred in Experiment A (2nd harvest) and Experiment B, but was absent from Experiment A (1st harvest) and Experiment C. It is presented in Table 5. The copper effect has been much smaller in calcium-deficient plants which can be satisfactorily explained by the lower growth rate and copper requirement of these plants.

The reason for the absence of interaction between calcium and copper in Experiment A first harvest is probably because calcium deficiency had not yet fully developed. Later, it would have been expected that the calcium effect would become much greater than that of copper as happened in the later harvest.

TABLE 5

p. 105. Table 5. The division line between "g dry weight" and "(log 10x)" should be deleted.

Experiment	ppm Cu	g dry wt.	(log 10x)
		Ca64	Ca8
A.2, whole plant	0.064	2.16	1.69
	0.0064	1.79	1.58
B, whole plant	0.064	1.57	1.23
	0.0064	1.39	1.19

Interaction of calcium, copper, and molybdenum

This contains within it the significant, first order interactions Ca × Cu already presented, and Ca × Mo, which can be discussed simultaneously with its highest order. Fig. 2 describes the interaction with respect to molybdenum levels. The response to molybdenum has been curious. With an adequate supply of calcium and copper there was no response to molybdenum, whereas at Cu 0.0064 there was increased growth where molybdenum was increased to 0.04.

This copper influence cannot be interpreted through its influence on growth for on these grounds alone the yield response to molybdenum would surely have occurred when growth was most vigorous, that is at Cu 0.064. Nor can the hypothesis of double deficiency be invoked here, since clearly there is no molybdenum deficiency.

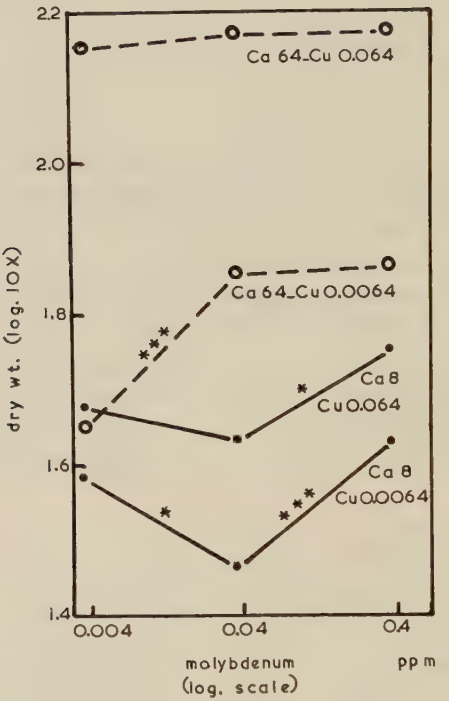


Fig. 2. Interaction of calcium, copper and molybdenum on yield (Exp. A. 2nd. harvest)

Under conditions of calcium deficiency, the results were no easier to interpret. Reference to Fig. 2 shows that at Ca8, smallest growth occurred at Mo 0.04 and that the yield has risen significantly whether the supply of molybdenum was increased tenfold or decreased tenfold.

The effects of molybdenum under calcium deficiency have occurred only in Experiment A, 2nd harvest. It has not been possible to repeat this.

Interactions involving phosphorus

It has already been noted that high levels of phosphorus inhibited yields especially when the nitrogen supply was small, and it can be seen from Table 4 that the response to phosphorus was influenced by the levels of calcium and copper in all experiments.

Interaction of calcium and phosphorus at different nitrogen levels. This became evident during growth as earlier and more severe symptoms of calcium deficiency where phosphorus levels were high. The ultimate effect is seen in Plate 3 and Fig. 3.

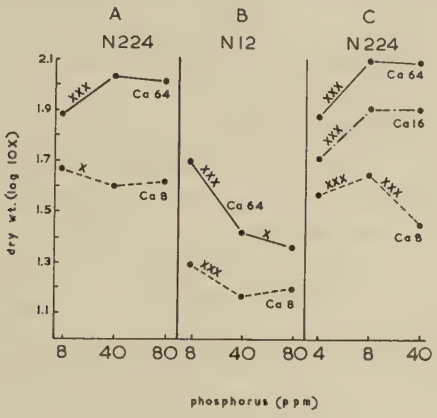


Fig. 3. Effect of nitrogen and calcium response to phosphorus

In each experiment, at Ca8, there was a negative response to an increase in phosphorus from P8 to P40, although this depression of yield was no greater at P80. Moreover, the yield response to phosphorus at Ca64 has depended on the nitrogen level. When this was high (Experiments A and C), P40 plants have grown as large or larger than P8 plants. That is, the Long Ashton level of phosphorus inhibited the growth of Mt. Barker if the nitrogen and/or the calcium supply were low.

Interaction of copper and phosphorus. This is presented in Table 6 and its general nature is such that wherever the level of phosphorus has inhibited yield on high copper, then the inhibition has been less or even absent on low copper. Thus the higher the copper level, the more readily did excess phosphorus affect yield adversely. This interaction is therefore of opposite sign to N × P

and Ca × P wherein increases in nitrogen and calcium relieved the toxicity.

Alternatively this interaction may be looked at from the point of view of copper responses at different phosphorus levels. In general it is seen (Table 6) that an increased copper supply has been most effective in increasing yields at the lowest phosphorus level (P4). Where toxicity was severe in Experiment A, 2nd harvest at P80 and Experiment B at P80 and P40, yields were the same at high and low copper.

In postulating a mechanism for the inhibition of growth by a moderately large phosphorus supply the following aspects must be considered: that low calcium and nitrogen levels, which induced poor yields, appeared to induce or intensify the toxicity; that the Long Ashton level of copper (even though it will be shown in a later paper to be sub-optimal, under a lower phosphorus regime), tended to intensify this toxicity, and that a lower copper regime, despite resultant poor growth tended to alleviate the toxicity. These facts will be discussed in the interpretation of chemical data later.

TABLE 6

Interaction of copper and phosphorus on yield

Experiment	ppm Cu	g dry weight (log 10x)				Components of means
		P80	P40	P8	P4	
A.1 (tops)	0.064	1.23 →	1.28 →	1.33	—	48
	0.0064	1.04 →	1.11 ←	1.02	—	
A.2 (whole plant)	0.064	1.91	1.95 ←	1.71	—	42
	0.0064	1.91 ←	1.71 ←	1.60	—	
B (whole plant)	0.064	1.25 →	1.30 →	1.64	—	42
	0.0064	1.28	1.26 →	1.33	—	
C (whole plant)	0.064	—	1.91 →	2.02 ←	1.91	42
	0.0064	—	1.70	1.72 ←	1.52	

CHEMICAL COMPOSITION OF TOPS

Where the yield is affected by the level of one of the variable elements, the concentration of the other elements may fall with vigorous growth due to growth dilution, and rise in inhibited plants. This is referred to hereafter as an *indirect* effect of a variable on

uptake. The critical point when looking for a *direct* effect, however, is whether the levels of a nutrient variable have influenced the uptake by other means than through yield, and to discover this, both concentration and total-uptake values must be studied together.

When the accompanying yield gradients have been large, it has been impossible to discover whether there has been any *direct* effect of factorial levels on the uptake of an ion, since the *indirect* influence of yield may have masked these manifestations. The simplest case is one in which factorial levels have not affected yield, for then a synergism is evident as increased concentration and absolute values, and an antagonism is evident as decreased concentration and total uptake. Nevertheless, where the yield gradient has not been large, synergism may also be evident as the maintenance of concentration against the dilution effect of growth. Whether or not this is apparent will depend on the extent of the yield differences and the strength of the synergism or antagonism.

Four replicate plants have been analysed for each nutrient treatment, with one determination per plant. Analyses of variance have been performed only on those levels of variables common to Experiments B and C so that comparisons may be made. In order not to exclude useful information for treatments not included in B and C, the results from such other treatments have been inserted in parentheses.

Nitrogen content of tops

Where nitrate has been the sole source of nitrogen (C) molybdenum levels have not influenced the nitrogen content of tops (Table 7). The changes in concentrations and absolute quantities of nitrogen in the plant brought about by varying the calcium and copper supply appear to be adjustments to large associated yield gradients. That is, increased yields have increased the total nitrogen of the plant but lowered concentration (arrows in opposite direction). In the symbiotic experiment (B), however, the higher levels of calcium and copper, which again produced superior yields of dry matter have also allowed a highly significant increase in nitrogen concentration. This suggests that calcium and copper have *directly* stimulated the fixation of nitrogen.

For sufficiency levels of phosphorus (P40 and P8) at N224, it

appears from Table 7 that the concentration of nitrogen has been maintained against a small yield gradient. At a sub-optimum level (P4) both relative and absolute amounts of nitrogen have been considerably reduced. This is in the range of positive yield response to phosphorus which is more usually encountered in agriculture.

TABLE 7

Nitrogen content of tops (32 components of means)				
Variables	Experiment C (N224)		Experiment B (N12)	
	% N	mg N/plant	% N	mg N/plant
Ca64	3.12	311	2.79	100
(Ca16)	(3.30)	(209)	—	—
Ca8	3.45	119	1.82	25
P40	3.30	202	2.44	48
P8	3.28	228	2.18	77
(P4)	(3.13)	(149)	—	—
Cu 0.064	3.24	268	2.44	82
Cu 0.0064	3.34	162	2.18	43
Mo 0.40	3.28	211	2.26	59
Mo 0.004	3.29	219	2.36	66

There was a highly significant increase in percent nitrogen in Experiment B at P40, and the smaller total production of nitrogen could be accounted for by the large phosphorus-induced growth inhibition. Consequently it does not appear that the development of phosphorus toxicity has *directly* limited the fixation of nitrogen. There was no P4 treatment in Experiment B and so the effect of insufficient phosphorus supply on symbiosis could not be observed.

These have been the overall effects of variables on nitrogen absorption and fixation. It is now necessary to study how these must be qualified as a result of interactions given in Table 8. All

TABLE 8

Significant interaction on nitrogen content of tops					
Interaction	Variance ratio		Interaction	Variance ratio	
	% N	mg/plant		% N	mg/plant
N × Ca	377 ***	58 ***	N × Ca × Cu	61 ***	—
N × Cu	28 ***	19 ***	N × Ca × P	—	27 ***
N × P	13 ***	—	Ca × Cu × P	5.5 *	—
Ca × Cu	7.9 **	16 ***			
Cu × P	—	12 ***	N × Ca × Cu × P	—	6.3 *

Levels of significance: * = 5%, ** = 1%, *** = 0.1%.

the significant interactions are contained in the third order interaction N × Ca × Cu × P which is presented in Table 9 with respect to copper and phosphorus effects on nitrogen content of tops.

TABLE 9

The interaction of nitrogen, calcium, copper and phosphorus on nitrogen content of tops

Variables			% N		mg N/plant		Yield (g)	
			Cu 0.064	Cu 0.0064	Cu 0.064	Cu 0.0064	Cu 0.064	Cu 0.0064
N224	Ca64	P40	2.97 →	3.29	382 ←	264 ↑	12.9	8.0
		P8	2.82 →	3.41	376 ←	222	13.3	6.5
	Ca8	P40	3.53	3.40	↓ 103 ←	58 ↓	2.9	1.7
		P8	3.63 ←	3.25	↓ 211 ←	102 ↓	5.8	3.1
N12	Ca64	P40	↑ 3.15 ←	2.73 ↑	↓ 79	60	2.5	2.2
		P8	2.86 ←	2.43 ↓	↓ 192 ←	68	6.7	2.8
	Ca8	P40	↑ 1.96	1.92 ↑	26	26	1.3	1.4
		P8	1.78	1.64 ↓	32	18	1.8	1.1

p. 111. Line 7. At the beginning of this line the words "greater and the concentrations" appear to have been omitted. [illegible] and total-nitrogen contents were lower at Cu 0.064, implying that the yield gradient was the principal factor affecting nitrogen uptake. At high nitrogen and *low calcium* (Ca 8) there has been an enhanced uptake of nitrogen at P40, Cu 0.064 but this has been more marked at P8.

Under the potentially active symbiotic system (N12–Ca64–P8) the decrease in nitrogen fixation was highly significant when copper was mildly deficient. Even under the restriction of phosphorus toxicity the copper effect on nitrogen fixation was still large. Further experiments which confirm this will be given in a later paper.

Phosphorus effects on nitrogen. By comparing nitrogen values and yield gradients at the two phosphorus levels, at any combination of the other variables it can be seen that the yield gradient has either accounted for the variation in nitrogen content or masked any direct effect of variation that may have taken place. Further, on the treatment N224–Ca64–Cu 0.064, both phosphorus levels produced the same vigorous growth but there was no significant difference in nitrogen uptake.

Calcium content of tops

Reference to Table 10 shows that all variables have significantly influenced the absolute and concentration values of calcium in the tops.

The high concentration of calcium in symbiotic plants was undoubtedly due to their slower growth rate. The higher calcium concentrations of calcium-deficient plants on N12, relative to N224, bear out the earlier suggestion that symptoms at N12 were those of nitrogen deficiency.

TABLE 10

Overall effects of controlled variables on calcium content of tops at two nitrogen levels (B/C)

Variables	N224		N12	
	% Ca	mg Ca/plant	% Ca	mg Ca/plant
Ca64	0.73 ↑	74 ↑	1.07 ↑	36 ↑
Ca8	0.24 ↓	9 ↓	0.42 ↓	6 ↓
Cu 0.064	0.48	53 ↑	0.78 ↑	26 ↑
Cu 0.0064	0.49	30 ↓	0.71 ↓	17 ↓
Mo 0.40	0.51 ↑	43 ↑	0.74	21
Mo 0.004	0.46 ↓	39 ↓	0.75	21
P40	0.48	40	0.84 ↑	19 ↓
P8	0.49	42	0.65 ↓	24 ↓

Copper has exerted a *direct* positive effect on the uptake of calcium but this was much more evident at N12 than N224. Molybdenum has directly influenced the calcium content, though this effect was slight and has occurred only at N224.

Phosphorus has had no *direct* effect on calcium uptake. This is seen in two ways. Firstly at N224 where increasing phosphorus from P8 to P40 had little effect on yield, there has been no difference in calcium content. Secondly, at N12 where P8 plants were much larger than those at P40, the concentration of calcium has decreased with increase of yield. The increased stress on Ca8 plants at high phosphorus levels hence cannot be explained as an effect on calcium uptake.

Several first- and second-order interactions occurred between N, Ca, P, and Cu but all these need qualification since the third-order

interaction of these variables was also significant with respect to percentage Ca. This interaction is presented in Table 11.

TABLE 11

Interaction of nitrogen, calcium, copper and phosphorus on calcium content of tops (8 components of means)

Variables			% Ca		mg Ca/plant		Yield (g)	
			Cu 0.064	Cu 0.0064	Cu 0.064	Cu 0.0064	Cu 0.064	Cu 0.0064
N224	Ca64	P40	0.70	0.75	94 ← « 56		13.0	7.5
		P8	0.72	0.76	94 ← « 51		13.0	6.7
	Ca8	P40	0.26	0.21	7	5	2.7	2.1
		P8	0.24	0.23	15 ← 8		6.3	3.5
N12	Ca64	P40	1.32 ← « 1.18		34	30	2.6	2.5
		P8	0.80 ≈→ 0.96		54 ← « 28		6.8	2.9
	Ca8	P40	0.51 ← « 0.36		7	5	1.3	1.4
		P8	0.49 ← « 0.33		8	4	1.7	1.3

Evidence of a *direct* positive effect of copper on the uptake of calcium is generally manifest. The occurrence of the lower order copper interactions mentioned above merely affect the magnitude of this copper effect. Thus, either at N224 or N12 wherever the yield gradient due to copper levels has been great, the percentage Ca has remained constant with the exception of the treatment N12–Ca64–P8; but in all cases, total uptake of calcium has been greater at Cu 0.064.

Phosphorus content of tops

It is to be noted in Table 12 that plants suffering from phospho-

p. 113. Lines 14 and 15 have been interchanged.

(N12–Ca64–Cu 0.064–P40) (heavy type) with their almost unaffected paring the phosphorus contents of the severely affected plants

TABLE 12

Phosphorus content of subterranean clover tops (8 components of means)

Levels of variables		N12		N224		Yield (g)	
		% P	mg P/plant	% P	mg P/plant	N12	N224
Ca64–Cu 0.064	P40	**1.23**	**34**	0.50	65	2.8	13.0
	P8	0.33	21	0.19	25	6.4	13.2
P40 — P8 = P-effect		0.90***	13***	0.31***	40***	—3.6	—0.2

N224 counterparts, the great increase in phosphorus concentration at P40 was not accompanied by the large increase in total uptake shown by the N224 plants. This suggests that it was the high concentration of phosphorus within the plant which was the prime factor causing the toxicity. Since such a suggestion would help to interpret the alleviating effects of added nitrate and calcium reported here and by Rossiter [15] as growth-dilution effects, it is useful to present the results of phosphorus analyses of tops as altered by the levels of phosphorus, calcium, nitrate, and copper. These are set out as the overall effects in Table 13. All variables

TABLE 13

Overall effects of variables on phosphorus content of tops

Variables	% P	mg P/plant
P40	1.06 ↑	33 ↑
P8	0.43 ↓	17 ↓
N224	0.55 ↓	30 ↑
N12	0.94 ↑	19 ↓
Ca64	0.60 ↓	32 ↑
Ca8	0.89 ↑	18 ↓
Cu 0.064	0.80 ↑	29 ↑
Cu 0.0064	0.69 ↓	20 ↓
Mo 0.40	0.73	24
Mo 0.004	0.76	25

have significantly influenced the percent of phosphorus of the tops except molybdenum but not in the same direction. Thus, the higher levels of nitrogen and calcium, which, it will be recalled, produced much greater yields than when in limited supply, have caused a great dilution of the phosphorus concentration. But the higher level of copper had the opposite effect. That is, despite the increased yield associated with this level of copper the phosphorus concentration was also greater. This represents a *direct* enhancement of phosphorus uptake by an increased copper supply.

This overall effect of copper on uptake of phosphorus requires qualification since copper interacted with all other variables to influence the phosphorus content of tops. It is most instructive to see how this copper effect behaved in the significant interaction

N × Ca × Cu × P which also contains most of the other significant interactions. The values for this interaction are set out with respect to decreasing copper effect on yield in Table 14.

TABLE 14

The interaction of nitrogen, calcium, copper and phosphorus on phosphorus content of tops

Levels of variables			% P		mg P/plant		Yield (g)
			Cu	Cu	Cu	Cu	Cu 0.064 — Cu 0.0064
N	Ca	P	0.064	0.0064	0.064	0.0064	= Cu effect
224	64	8	0.19 →	→ 0.29	25 ←	← 18	13.2 — 6.5 = 6.7
224	64	40	0.50 →	→ 0.66	65 ←	← 52	12.9 — 7.3 = 5.6
224	8	8	0.44	0.46	23 ←	← 13	5.8 — 2.1 = 3.7
12	64	8	0.33 →	→ 0.47	21 ←	← 12	6.3 — 2.7 = 3.6
224	8	40	0.97	0.91	29 ←	← 16	2.9 — 1.7 = 1.2
12	64	40	1.23	1.16	34 ←	← 25	2.9 — 2.2 = 0.7
12	8	8	0.75 ←	← 0.53	12 ←	← 7	1.8 — 1.2 = 0.6
12	8	40	1.99 ←	← 1.05	25 ←	← 14	1.3 — 1.3 = 0.0

The heavy line divides the non-toxic combinations of variables above, from the toxic below. Moreover, (far right-hand column) this line separates those treatments where the effect of copper levels on growth was large, from the small or non-existent. It can be seen that where the copper levels caused large yield differences these have indirectly influenced the phosphorus concentration and total uptake values, *i.e.* gradient arrows for concentration are in the opposite direction to the arrows for total phosphorus. Here it is impossible to detect any direct synergism between copper supply and phosphorus content. But where the yield effect of copper was small or absent, then, both concentration and total uptake of phosphorus in tops were enhanced (arrows in the same direction). This enhanced phosphorus concentration at Cu 0.064 becomes more evident as one proceeds down the table. It therefore seems reasonable to suggest that the synergistic effect of copper on phosphorus uptake which is so strongly in evidence where yield differences are small, also occurs where the differences are very large, but that it is masked; that is to say, the plant was unable to maintain its phosphorus concentration against such a yield gradient. Finally, the phosphorus concentration of plants suffering from phosphorus toxicity was in general much higher than on those not showing such effects.

The foregoing results suggest that the cause of phosphorus toxicity was a high concentration of phosphorus in the tissue rather than in the nutrient solution. Further, when the level of one or more of the nutrients was sufficiently low to inhibit growth, and the supply of phosphorus was generous, then the resultant high phosphorus concentration in the plant may have been sufficient to cause further depression of growth and even necrosis.

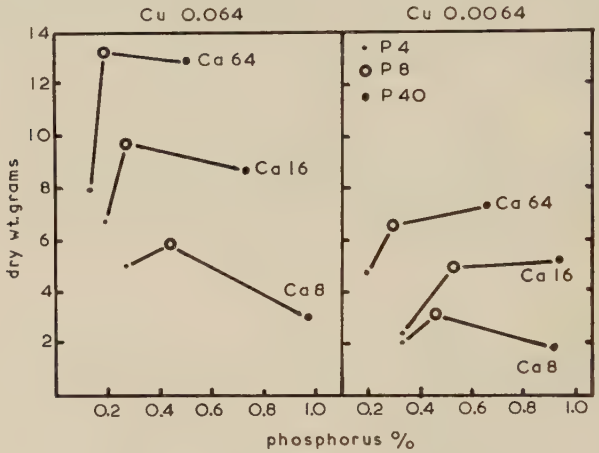


Fig. 4. Relation between yield and phosphorus concentration of tops (Exp. C)

This relationship between the yield (or perhaps better, growth rate) and the percent P of tops of Mt. Barker can be seen in Fig. 4. This data was taken from Experiment C containing the treatment P4, which was insufficient for optimum growth. Starting with the highest yielding "trio" of P levels one sees the great yield response from P4 to P8, but as the yields have decreased due to another limiting factor this response became smaller, due according to this suggestion, to the rise in the phosphorus concentration. Between P8 and P40 the effect of decreasing yield has shown the same trend in percentage P so that the highest yield allowed no response to the P40 treatment, but as the concentration has risen a distinct negative response occurred. It appears that above 0.6 per cent P, there was a slight growth inhibition, which became severe beyond 0.9 per cent P. Large positive responses occurred only below 0.3 per cent P. Under a low-copper regime all these critical concentrations were higher.

It has been shown (Table 13) that the overall value of phosphorus concentration was slightly higher at Mo 0.004, although there was no significant effect on total uptake. This effect of molybdenum levels on phosphorus concentration has interacted with N, Cu and P levels. On reference to the interaction N × Cu × Mo × P which contained all these lower-order interactions within it, it was found that the significantly increased concentrations of phosphorus occurred only under symbiosis and at P40. This part of the interaction is tabulated in Table 15.

TABLE 15

Effect of molybdenum levels on phosphorus content of tops (8 components of means)

Variables	% P		mg P/plant		Yield (g)	
	Mo 0.04	Mo 0.004	Mo 0.04	Mo 0.004	Mo 0.04	Mo 0.004
N12–Cu 0.064–P40	1.50 ↘	→ 1.71	33 ←	27	2.4	1.8
N12–Cu 0.0064–P40	1.02 ↘	→ 1.18	16 →	23	1.8	1.9

In summary of these effects of controlled variables on the phosphorus content of subterranean clover, it can now be said: (i) that nitrate nitrogen and calcium exerted an indirect influence by virtue of their yield effects; (ii) that a near-optimum level of copper has directly enhanced the uptake of phosphorus compared with a sub-optimal level, but being more noticeable when the yield effects were small, and (iii) that molybdenum levels have had little or no influence on phosphorus uptake.

Copper content of tops

From Table 16 it is seen that at either nitrogen level the concentration of copper was much lower, and the total uptake much higher at Ca64 than Ca8. These differences are adequately explained by the large growth effects.

For the N224 group, the concentration of copper was significantly higher at Mo 0.40, but the total uptake of copper was not significantly different. With the N12 plants the concentrations of copper were higher at Mo 0.004, but again there was no significant difference in total content. Yield differences again account for these variations in copper content.

TABLE 16

Overall effect of controlled variables on copper content of tops of plants grown on high copper (16 components of means)

Variables	ppm Cu		Microgram Cu/plant		Yield (g)	
	Ca64	Ca8	Ca64	Ca8	Ca64	Ca8
N224	8.1 →	16.7	107 ←	75	13.0	4.8
N12	19.7 →	29.1	80 ←	44	4.1	1.6
	Mo 0.40	Mo 0.004	Mo 0.40	Mo 0.004	Mo 0.40	Mo 0.004
N224	13.8 ←	11.0	95	86	8.9	9.0
N12	21.4 →	27.4	59	65	3.2	3.1
	P40	P8	P40	P8	P40	P8
N224	14.1 ←	10.7	89	92	8.2	9.7
N12	27.0 ←	21.8	48 →	76	1.9	4.4

However, further light can be thrown into the Cu–Mo relationship by the significant interaction Ca × Mo × P for N224 plants. This is presented in Table 17, where it is seen that for the combination of Ca8 and P40, both concentration and total uptake were significantly greater at the higher molybdenum level. This must be regarded as enhanced uptake of copper caused by a large increase in molybdenum supply. A similar effect is seen for the combination Ca64–P8, but in general this series of results does not offer good evidence of any *direct* influence of molybdenum level on uptake of copper, when the copper is in good supply.

TABLE 17

Interaction of calcium, molybdenum and phosphorus on copper content of tops (4 components of means)

Variables	ppm Cu				Microgram Cu/plant			
	P40		P8		P40		P8	
	Mo 0.40	Mo 0.004	Mo 0.40	Mo 0.004	Mo 0.40	Mo 0.004	Mo 0.40	Mo 0.004
Ca64	8.4	9.15	8.1	6.8	108	121	111	87
Ca8	24.2 ←	14.7	14.5	13.4	77 ←	50	83	88

The influence of phosphorus on copper content of tops of the high copper plants can be seen in Table 16. These differences, though substantial, are *indirect* effects of yield. In Table 18, however, where a few copper values are given for Cu 0.0064 plants it is evident that P40 treatments have induced a considerable

increase in both total uptake and concentration of copper. The same deduction can be made also from Table 17, especially under calcium-deficiency conditions.

The copper concentration of Cu 0.0064 plant tops was not unduly low, which corresponded with the mildness of the deficiency. Moreover, since the plants were limited by copper, it would be expected that the concentration values would be less affected than the absolute values.

TABLE 18

Copper content of plant tops on a low copper supply (Cu 0.0064)

Nutrient treatment		ppm Cu	Microgram Cu/plant	Components of means
N224–Ca64	P40	6.8	50	6
	P8	5.9	42	6
N12–Ca64	P40	8.6	21	5
	P8	4.6	12	5

Molybdenum content of tops

It is seen from Table 19 that P40 has enhanced the uptake of molybdenum from those levels not supplying molybdenum in gross amounts (0.04 and 0.004 ppm). Such a supply is of the order available in many soils. Under these conditions, both concentration and total uptake of molybdenum were greater at P40

TABLE 19

Effect of copper and phosphorus on molybdenum content of tops (4 components of means)

Variables			ppm Mo		Microgram Mo/plant		Yield (g)	
			Cu 0.064	Cu 0.0064	Cu 0.064	Cu 0.0064	Cu 0.064	Cu 0.0064
N12	Mo 0.40	P40	30.7	40.6	80	76	2.6	1.9
		P8	22.3	47.4	142	143	6.3	3.0
	Mo 00.4	P40	11.7	11.7	26.5	18	2.3	1.5
		P8	2.4	3.5	10.5	12	4.4	3.4
	Mo 0.004	P40	4.8	6.0	12.4	13.1	2.6	2.0
		P8	1.1	1.4	6.7	7.9	6.1	5.6
N224	Mo 0.40	P40	14.4	14.8	184	99	12.9	7.1
		P8	12.5	12.8	170	90	13.6	7.1
		P4	10.6	12.4	88	70	7.6	5.8
	Mo 0.004	P40	2.8	1.1	36	9.5	13.2	9.1
		P8	1.7	2.5	23	15	12.8	6.0
		P4	0.65	0.7	5	3	7.9	4.3

despite the very large yield gradient against it produced by phosphorus toxicity. For example, for plants receiving 0.04 ppm molybdenum, the P40 plants are only half the size of P8 plants, yet contain twice as much molybdenum. Where gross amounts of molybdenum were supplied (Mo 0.40), there has been no *direct* effect of phosphorus level on uptake.

At N224, where growth was much more vigorous than under symbiosis, the demand for molybdenum was greater, and generally the concentrations of molybdenum were much lower. Under these conditions, the effect of increasing the phosphorus supply has again been to enhance the uptake of molybdenum even at Mo 0.40.

The effect of copper is not so clear cut. At N12 there appears to be no *direct* effect of copper on uptake of molybdenum, but at N224, molybdenum concentrations have not been affected by copper levels, even though 0.064 ppm of copper has produced much larger plants.

DISCUSSION

Effects of calcium and copper on symbiosis

It is quite clear that a given nutrient disorder cannot be conceived of as acting independently on either host plant or rhizobial activity, since the activities of one affect the other. However, from the nature of the results presented here, it is possible to judge whether the direct or primary effect of a controlled variable has been on the host plant, the nitrogen fixation mechanism, or both. It is not possible though, to distinguish which aspect of nodular activity has been affected since the appearance of nodules is too empirical a guide, and also, poor fixation may be due to slow utilisation of end products as much as to low fixation potential.

The results presented in this paper are limited to the comparison of dry-weight data from symbiotic and a-symbiotic clover plants along with appearance of nodules and Kjeldahl-nitrogen values expressed as total nitrogen for plant tops and as concentrations. Any interpretation therefore must be in terms of these relatively crude criteria. The use of Kjeldahl values as an index of nitrogen fixation achievement has obvious limitations. It is impossible to distinguish the separate contribution of the nodules from the small amount of absorbed nitrate which is rapidly reduced within the

plant. Further, it measures all reduced nitrogen present irrespective of whether or not it has been fully incorporated into the metabolic cycle. In cases where a nutritional disorder blocks entry of fixed nitrogen into normal paths, rather than blocking the initial fixation of nitrogen, this weakness could be serious.

The importance of calcium for successful nodulation has been well established for temperate legumes, but recently Norris [11] has drawn attention to the low calcium requirements of free-living rhizobia. Since results presented here show clearly that calcium deficiency in a predominantly symbiotic system appears as nitrogen deficiency of the top and necrosis and reduction in size of the nodule, it seems that the limiting factor in nitrogen fixation when calcium is in short supply is in the failure of the plant to develop healthy nodulation, rather than the demands of the rhizobia themselves, as has also been shown (Loneragan [24]).

The stimulation of nodules and nitrogen content for symbiotic plants by copper has already been reported (Hallsworth [8]), but the evidence presented for it requires qualification. It has been shown (Tables 7 and 9) that over a certain range of copper supply the fixation of nitrogen could be greatly enhanced by this nutrient. Moreover, when this occurred the yield also was greatly increased. This is an important point because at N12, the response to copper was relatively small over those ranges where nitrogen fixation was not enhanced. In other words, where either the nitrogen supply was generous, or fixation vigorous, subterranean clover responded strongly to a tenfold increase in copper supply; but where nitrogen supply and fixation were low, the response to copper was poor. This is not difficult to understand, since within very broad limits, the better the nitrogen status of the plant, the more rapidly and larger will it grow, and hence the greater becomes the copper demand, and a tendency for the copper concentration of the plant to fall has been shown. As the overall copper concentration of N12 plants was twice that of N224 plants, obviously growth responses would not be so great unless nitrogen fixation was accelerated.

It will be recalled that when subterranean clover was grown on a low nitrogen and excessive phosphorus supply (N12–P40) the nitrogen fixation was severely limited as compared with plants at P8. But since the increase in nitrogen concentration at P80 was

highly significant, there is the possibility that utilization of nitrogen may have been inhibited as suggested by Williams [21] who observed reduced quantities of phospho-proteins in high-phosphorus oat plants.

Phosphorus toxicity

The adverse effects of excess phosphorus have been frequently reported in plant nutrition studies as an unexpected result. However, Shive [17] using soybean, was probably the first to study this problem and its designation as a toxicity was given by Hammer. Investigations on the phosphorus requirement of vegetables by Woodman [22]and Woodman and Johnson [23] demonstrated that cabbage and turnip, for example, did not respond positively to increases in phosphorus in cultures beyond about 6 ppm. Their data show a very narrow optimum range above and below which yields declined sharply. More recently Rossiter [15 16] demonstrated depressing effects of moderately large dressings of superphosphate on Dwalganup subterranean clover when grown in a non-ferruginous sandy soil. Such field conditions are not far removed from those of sand culture. The alleviating effects of both nitrate and calcium observed in the present experiments were also noted by him. Thus whether a given supply of phosphorus is inadequate, sufficient, or excessive depends on the growth rate as limited by other environmental factors. Conversely the concentration of phosphorus in a plant is a function not only of the available supply of phosphorus to the roots but also of growth rate, particularly in the early stages. From this work it is clear that the calcium, nitrogen, copper, and molybdenum concentrations of the tops can also be varied as much by altering the levels of other nutrients in the medium as by varying the supply of the particular element itself. This places grave limitations on the use of chemical data of plants as an index of the supply of a given nutrient in the medium when expressed as a concentration only. In the special case of the symbiotic plant, growth rate may be grossly affected by the absence of effective rhizobia, a factor which may bear no relation to the nutrient status of the soil and yet which could cause a relatively small supply of available phosphorus to build up a concentration in the top which would give a misleading index of that small supply. Indeed, Rossiter suggested that the

beneficial effect of added calcium in his field experiments with phosphorus toxicity was due to the stimulation of nodulation.

On first consideration it might be thought that phosphorus toxicity could be due to the fixation of a nutrient either by co-precipitation or by irreversible adsorption on to a phosphate precipitate. However, all the evidence is against this. In the first place, though the uptake of a nutrient such as calcium certainly did not become a limiting factor, it was shown that on the highest nitrogen level the concentration of this element was considerably less than on low nitrogen supply where toxicity was most severe. Secondly, the symptoms were not those of iron deficiency, either in appearance or the age of leaf affected. That is, the oldest leaves suffered and not the youngest as in true iron deficiency. Thirdly, any nutrient shortage due to precipitation would have been more acute in the most vigorously growing plants *i.e.* on N224–Ca64 treatments. But these were the plants least affected by high phosphorus levels. This negative evidence gives further indirect support to the idea that phosphorus toxicity is due to events within rather than at the root surface.

Rediske and Biddulph [14], after showing that high phosphorus in the nutrient did not prevent the entry of iron from ferric citrate providing it was in reasonable supply, proceeded to study the distribution of this iron in the leaves of kidney beans by radio autograph. On a high phosphorus supply, iron was concentrated in the veins, and the inter-veinal areas developed chlorosis. They suggested that high phosphorus in the leaf precipitated iron locally in the vein. However, phosphorus toxicity in subterranean clover did not always induce marked interveinal chlorosis but rather depressed growth, which was followed suddenly by necrosis in severe cases. The necrosis is seen to work back through the inter-veinal areas and P80 plants show three leaves with well defined interveinal necrosis. This confirms the observations of Rossiter.

The possibility that there is a breakdown in metabolism is rendered more conceivable by the work of Brown and Holmes [4], Brown, Holmes and Specht [5] and Brown, Holmes, Shapiro and Specht [6], who measured catalase activity in several species grown on calcareous soil and which became chlorotic when phosphorus and copper were added together or separately. They found that active iron (catalase activity) decreased as the phosphorus content

of the soil was raised and that large additions of copper intensified this. Such an effect of copper intensified phosphorus toxicity in spite of the accompanying growth dilution. For the synergistic effect of copper on phosphorus uptake, though significant, was so small that it could not account for the great effect on phosphorus toxicity once this had been induced. It seems more likely that the high concentration of phosphorus precipitates much of the iron, possibly in the veins, so reducing catalase activity to a critically low level, and that a relatively high copper concentration (in the order of 20 ppm in the top) may then have a dramatic effect. Whereas in low-copper plants, although the phosphorus concentration became high, the copper concentration rarely attained 10 ppm and was more often about 5 ppm or less, which may be inocuous in this respect.

Copper and molybdenum contents

From Tables 16 and 19, which dealt with copper and molybdenum contents of tops respectively, it will be realised that all the controlled variables have substantially influenced the total quantities and/or concentrations of these two elements. Some have achieved this by altering the growth rate, and in general, such effects have been second only to the supply of the element in altering the concentration in the plant. Taking as example the concentrations of molybdenum at P40 and Cu 0.064 (from Table 19):

Variables	N224	N12
Mo 0.4	14.4 ppm	30.7 ppm
Mo 0.04	—	11.7 ,,
Mo 0.004	2.8 ,,	4.8 ,,

it can be seen that retardation of growth caused by low nitrogen supply doubled the molybdenum concentration. When the molybdenum supply was generous, this increase brought the molybdenum concentration in the top to a value dangerous to stock. Furthermore, under such slower growing conditions, the synergistic influence of phosphorus on molybdenum uptake was greater, and whether or not this was due to a further depression of yield by phosphorus is of little importance to animal nutrition. These remarks confirm the observations of Barshad [1 2] on the effect of growth rate on uptake of molybdenum.

Similarly with copper (Table 16) shortage of nitrogen has caused copper concentrations to be doubled and calcium deficiency has produced the same trend. If these changes are compared with the critical values of Teakle and Turton [20], it is seen that the plants on the same copper supply may exist in several of their categories, depending on the nutritional treatment.

It will be recalled from Table 19 that phosphorus greatly enhanced the uptake of molybdenum when present in a moderately low supply in the nutrient solution. This confirms the results of Stout and Meagher [18] and Stout *et al.* [19] for tomato, and Barshad [3] for ladino clover. When copper was limited so that P40 was not inhibiting growth, it was seen (Table 18) that both concentration and total uptake of copper was also enhanced by phosphorus. The magnitude of this Cu–P synergism with a copper supply of 0.0064 ppm is of the same order as the Mo–P synergism. Consequently, the ratio of Cu/Mo has not been greatly altered by increasing the phosphorus supply. These ratios are given in Table 20 where it is seen that the largest difference is very small compared to those induced seasonal changes given by Piper and Beckwith [12]. Again applying Teakle and Turton's copper values to the phosphorus effect in Table 19 (4.6 ppm Cu to 8.6 ppm Cu) it is seen that the copper status of the plant has been increased from "low normal" to "high normal" and, with respect to stock, it has passed from "deficiency" to "sufficiency", without altering the copper supply.

In this work the yield effect of variables has been used as a means of interpreting chemical data rather than the reverse. This has been an unforseen benefit from the factorial design, without which interpretation of *direct* and *indirect* nutrient interactions would have been impossible. It is suggested that this aspect of the factorial design, in presenting chemical interactions over a range of growth rates is worthy of more attention.

SUMMARY

A study has been made of specific effects and interactions of Ca, Cu, Mo, and P on the symbiotic system and chemical composition of subterranean clover, (Mt. Barker var.) grown in sand culture under glass on high and low nitrate supply. The levels of Ca and Cu were low while those of Mo and P were in general high or excessive.

N_2-fixation appeared to be specifically inhibited by deficiencies of Ca or Cu but was only affected by Mo or P according to their effect on yield. The classic symptoms of Ca- and Cu-deficiencies were obtained on high-nitrate plants, but in symbiotic plants they appeared as symptoms of N-deficiency. This was confirmed by chemical analysis.

The main yield interactions were Ca × Cu, Ca × P and Cu × P which were explained by the chemical data. Toxic effects of excess P which were observed were relieved by the increased growth rate associated with higher levels of nitrate or Ca, but were intensified by increased levels of copper. Higher levels of nutrient-P enhanced equally the Cu- and Mo-content of tops.

Copper deficiency depressed the uptake of Ca into the tops.

ACKNOWLEDGEMENTS

The authors are indebted to Mr. E. May and Miss H. Lee for technical assistance and to Mr. F. C. Treeby for the photographs.

Received January 28, 1959.

BIBLIOGRAPHY

1 Barshad, I., Molybdenum content of pasture plants in relation to toxicity to cattle. Soil Sci. **66**, 187 (1948).
2 Barshad, I., Factors affecting the molybdenum content of pasture plants: I. Nature of soil Mo, growth of plants and soil pH. Soil Sci. **71**, 297 (1951).
3 Barshad, I., Factors affecting the molybdenum content of pasture plants: II. Effect of soluble phosphate, available nitrogen and soluble sulphate. Soil Sci. **71**, 387 (1951).
4 Brown, J. C. and Holmes, R. S., Iron, the limiting element in a chlorosis: Part I. Availability and utilization of iron dependent upon nutrition and plant species. Plant Physiol. **30**, 451 (1955).
5 Brown, J. C., Holmes, R. S. and Specht, A. W., Iron, the limiting element in a chlorosis: Part II. Copper-phosphorus induced chlorosis dependent upon plant species and varieties. Plant Physiol. **30**, 457 (1955).
6 Brown, J. C., Holmes, R. S., Shapiro, R. E. and Specht, A. W., Effects of phosphorus and copper on iron chlorosis of rice in flooded and non-flooded soil and the associated enzymatic activity. Soil Sci. **79**, 363 (1955).
7 Greenwood, E. A. N. and Hallsworth, E. G., An evaluation of the maximum radius of the rosette as a growth index for subterranean clover. Plant and Soil **12**, 49–56 (1960).
8 Hallsworth, E. G., Nutritional factors affecting nodulation. Proc. Univ. Nottingham 5th. Easter School in Agr. Sci. (Ed. E. G. Hallsworth). Butterworths Scientific Publications, London, p. 183 (1958).
9 Hewitt, E. J., Sand and water culture methods used in the study of plant nutrition. Commonwealth Bur. Hort. Plantation Crops Tech. Common. **No. 22** (1952).
10 Millikan, C. R., Nutritional disorders in subterranean clover. Dept. Agr. Victoria Tech. Bull. **No. 11** (1953).
11 Norris, D. O., *Rhizobium* needs magnesium not calcium. Nature **182**, 734 (1958).
12 Piper, C. S. and Beckwith, R. S., Uptake of copper and molybdenum by plants. Proc. Spec. Conf. Plant Animal Nutrition Australia 1949, 144 (1951).

13 Purvis, E. R. and Peterson, N. K., Methods of soil and plant analysis for molybdenum. Soil Sci. **81**, 223 (1956).
14 Rediske, J. H. and Biddulph, O., Adsorption and translocation of iron. Plant Physiol. **28**, 576 (1953).
15 Rossiter, R. C., Phosphorus toxicity in subterranean clover and oats grown on Muchea sand, and the modifying effects of lime and nitrate nitrogen. Australian J. Agr. Research **3**, 227 (1952).
16 Rossiter, R. C., The influence of soil type on phosphorus toxicity in subterranean clover. Australian J. Agr. Research **6**, 1 (1955).
17 Shive, J. W., Toxicity of monobasic phosphates towards soybeans grown in soil and solution culture. Soil Sci. **5**, 87 (1918).
18 Stout, P. R. and Meagher, W. R., Studies of molybdenum nutrition of plants with radio molybdenum. Science **108**, 471 (1948).
19 Stout, P. R., Meagher, W. R., Pearson, G. A., and Johnson, C. M., Molybdenum nutrition of crop plants I. The influence of phosphate and sulphate on the absorption of molybdenum from soils and solution cultures. Plant and Soil **3**, 51–87 (1951).
20 Teakle, L. J. H. and Turton, A. R., The copper, manganese and zinc content of subterranean clover and oats in Western Australia. J. Dept. Agr. Western Australia **20**, 238 (1943).
21 Williams, R. F., The effect of phosphorus supply on the rate of intake of phosphorus and nitrogen upon certain aspects of phosphorus metabolism in gramineous plants. Australian J. Sci. Research Ser. B **1**, 333 (1948).
22 Woodman, R. M., Nutrition of vegetables in sand. Ann. Appl. Biol. **31**, 22 (1944).
23 Woodman, R. M. and Johnson, D. A., Plant growth with nutrient solutions. IV. J. Agr. Sci. **37**, 163 (1947).
24 Loneragan, J. F., Calcium in the nitrogen metabolism of subterranean clover. Australian J. Biol. Sci. **12**, 26–39 (1959).

Plant and Soil XII, no. 2 March 1960

A RELATION BETWEEN THE RATIOS OF PHOSPHORUS TO IRON AND POTASSIUM TO CALCIUM IN MUSTARD LEAVES

by P. C. DEKOCK, A. HALL and MARGARET MCDONALD

Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen

INTRODUCTION

That chlorotic leaves may contain higher concentrations of phosphorus than green leaves has been shown by the studies of Olsen [29] and Ehrenberg [17]. In an investigation into the effect of various forms of complexed iron on the phosphorus and iron content of leaf ash of mustard plants grown in nutrient culture DeKock and Strmecki [14] proposed that the ratio of total phosphorus to iron in the ash of leaves could be used to assess iron-deficiency chlorosis rather than total iron, chlorotic leaves having higher ratios than green leaves.

From the results of a great number of fertilizer experiments, Ehrenberg [16] concluded that the relative amounts of potassium and calcium supplied were important in determining crop yield. Lundegårdh [25] in his book on leaf analysis points out that the significance of this ratio of potassium to calcium was the most important fact emerging from his studies of the mineral composition of leaves over many years. This potassium/calcium ratio has been used as an indicator of lime-induced chlorosis by Lindner and Harley [23] and by Bennett [3] although the latter pointed out that it was not always found to be in accordance with the symptoms.

From the results of Lindner and Harley [23] and Bennett [3] it could be seen that both the P/Fe and K/Ca ratios were greater in chlorotic leaves than in green leaves. Observations of the former authors on genetical chlorosis in *Spiraea* were extended to include a great number of genera (DeKock and Hall [12], and unpublished)

with the finding that the P/Fe and K/Ca ratios were always higher in the chlorotic leaves.

Since the mineral constitution of a normal green plant is affected by the composition of the nutrient solution, it follows that if the above ratios were maintained by a metabolic balance in the leaf, alteration in one ratio would necessarily imply a corresponding alteration in the other.

Where phosphate concentration in the nutrient solution is high, iron is usually precipitated, and so is unavailable to the plant (Biddulph and Woodbridge [4]). Consequently chelating agents such as ethylene diamine tetra-acetic acid (FeV) and ethylene N,N′-*bis*(2-*o*-hydroxyphenyl) glycine (FeG) have been employed to maintain the iron in solution. Experiments were therefore designed using mustard plants in nutrient culture with different ratios of phosphorus to nitrogen and potassium to calcium and three levels of iron supplied as $FeCl_3$, FeV and FeG.

METHODS

Mustard plants were grown in 5-litre glazed crocks, the plants supported on an all-plastic circle of gauze held by aluminium nails and containing a little washed silica sand, seedlings being thinned to 15 per crock when the cotyledons had expanded.

Nutrient solutions (Table 1) were chosen to give 4 ratios of potassium to calcium and 2 ratios of phosphorus to nitrogen so that the sum of cations and the sum of anions remained unaltered. Iron was supplied at 3 levels

TABLE 1

Nutrient solutions (milliequivalents/litre)							
Treatment	$Ca(NO_3)_2$	$CaSO_4$	K_2SO_4	$MgCl_2$	Na_2HPO_4	$NaNO_3$	KNO_3
K_1P_1	10	2	1	2	1	1	—
K_2P_1	10	—	3	2	1	1	—
K_3P_1	7	—	3	2	1	1	3
K_4P_1	3	—	3	2	1	1	7
K_1P_2	10	2	1	2	2	—	—
K_2P_2	10	—	3	2	2	—	—
K_3P_2	7	—	3	2	2	—	3
K_4P_2	3	—	3	3	2	—	7

viz 0.2, 1.0, and 5.0 ppm. Sodium, magnesium, chloride and sulphate ions were supplied in constant amounts and a solution of micro-nutrients added to give 1 ppm Mn, 0.1 ppm Cu, 0.05 ppm Zn, 0.02 ppm B, 0.02 ppm Mo, and 0.001 ppm V. The treatments were arranged in a 4 × 3 × 2 factorial design.

Each treatment was repeated twice and the treatments randomised in 2 blocks of 24. Solutions were renewed when the plants were half grown at which time less than one tenth of any major nutrient had been absorbed. The pH was adjusted to 5.5 on alternate days and the solutions aerated for 8 hours daily.

Harvesting took place as soon as flower buds showed in the most advanced treatments. The plants were scored for chlorosis on a decimal scale of yellow to green then severed just above the supporting ring. The leaves were separated from the stems and dried at 90°C for 48 hours then ashed at 460° overnight and the ash analysed for iron, phosphorus, calcium, magnesium, potassium and sodium as in previous studies (DeKock and Hall [12]). Three experiments, with iron supplied as $FeCl_3$, FeV and FeG were carried out.

RESULTS

1. *Visual effects* (Tables 2 and 3).

(a) $FeCl_3$ experiment. All treatments grew very poorly when iron was supplied as ferric chloride and the majority of plants at the higher phosphorus level (P_2) died or made no growth beyond the first true leaves. At the lower (P_1) level there was a marked effect of the highest iron level on the growth and colour of the plants but these Fe_3 plants were still comparatively small averaging only 8.6 cm in height. Little difference could be seen between the Fe_1 and Fe_2 levels, plants being small and highly chlorotic.

Increasing the level of K (*i.e.* increasing the K/Ca ratio) in the nutrient solution caused growth in height and weight to decrease and led to an intensification of the symptoms of chlorosis. At the higher P level, only the K_1 and K_2 plants survived.

(b) FeV experiment. Presence of the EDTA chelate caused a very pronounced response in growth at the Fe_2 and Fe_3 levels whereas the Fe_1 plants were small and chlorotic. Phosphorus level only significantly affected height at the K_1 level, increase in P causing a decrease in height. Increase in K level caused a decrease in height, but had no significant effect on colour.

(c) FeG experiment. Presence of the phenolic chelate caused good growth at even the lowest iron level and the experiment was characterised by the extreme uniformity of all treatments. All plants were green, though a tendency to yellowing was visible at the lowest iron level. (The Fe_1 plants were also somewhat smaller than the Fe_2 or Fe_3 plants). Phosphorus level again showed no

TABLE 2

Average height (cm) of mustard plants grown at 3 levels of iron, 4 levels of K and 2 levels of P

Treatment	Fe_1	Fe_2	Fe_3	P_1	P_2	Mean
$FeCl_3$ experiment						
		(± 1.59)		(± 1.30)		(± 0.92)
K_1	9.2	9.5	13.5	11.8	9.7	10.8
K_2	6.0	6.5	9.8	10.5	4.3	7.4
K_3	5.8	5.0	5.2	7.3	3.3	5.3
K_4	4.5	2.8	6.0	5.2	3.7	4.4
		(± 1.12)				(± 0.65)
P_1	7.1	6.8	12.2			8.7
P_2	5.6	5.1	5.0			5.2
Mean	6.4	5.9	8.6			7.0
		(± 0.79)				
FeV experiment						
		(± 1.39)		(± 1.14)		(± 0.81)
K_1	8.2	16.8	16.5	15.8	11.8	13.8
K_2	7.0	15.0	20.5	13.7	14.7	14.2
K_3	7.5	16.2	20.0	14.7	14.5	14.6
K_4	6.8	12.8	16.0	11.8	11.8	11.8
		(± 0.99)				(± 0.57)
P_1	7.9	15.8	18.4			14.0
P_2	6.9	14.6	18.1			13.2
Mean	7.4	15.2	18.2			13.6
		(± 0.70)				
FeG experiment						
		(± 1.57)		(± 1.29)		(± 0.91)
K_1	30.2	33.5	32.8	32.7	31.7	32.2
K_2	29.0	33.2	32.0	30.8	32.0	31.4
K_3	29.8	30.0	32.2	30.3	31.0	30.7
K_4	29.0	28.8	29.8	30.3	28.0	29.2
		(± 1.11)				(± 0.64)
P_1	29.6	32.2	31.2			31.0
P_2	29.4	30.5	32.1			30.7
Mean	29.5	31.4	31.7			30.9
		(± 0.79)				

significant effect on height or colour but increase in potassium level caused a decrease in height.

2. *Weight measurements*

(a) $FeCl_3$ experiment. Growth of plants was so poor that only at the highest iron level and the lowest P level was growth at all significant. Hence both fresh and dry weights of whole plants, leaves, and stems showed an increase at the highest iron level and

TABLE 3

Colour of leaves of mustard plants on decimal scale of increasing greenness						
Treatment	Fe_1	Fe_2	Fe_3	P_1	P_2	Mean
$FeCl_3$ experiment						
		(± 0.30)		(± 0.25)		(± 0.18)
K_1	3.5	3.8	4.5	4.3	3.5	3.9
K_2	2.5	2.5	3.5	3.7	2.0	2.8
K_3	1.8	2.0	2.8	3.3	1.0	2.2
K_4	1.8	1.5	1.8	2.3	1.0	1.7
		(± 0.22)				(± 0.12)
P_1	2.9	3.0	4.4			3.4
P_2	1.9	1.9	1.9			1.9
Mean	2.4	2.4	3.1			2.7
		(± 0.15)				
FeV experiment						
		(± 0.46)		(± 0.37)		(± 0.26)
K_1	2.2	5.2	7.5	5.2	4.8	5.0
K_2	2.5	4.5	8.0	5.0	5.0	5.0
K_3	2.0	5.8	7.8	5.0	5.3	5.2
K_4	1.2	4.2	8.0	4.7	4.3	4.5
		(± 0.32)				(± 0.19)
P_1	2.1	4.9	7.9			5.0
P_2	1.9	5.0	7.8			4.9
Mean	2.0	4.9	7.8			4.9
		(± 0.23)				
FeG experiment						
		(± 0.44)		(± 0.36)		(± 0.26)
K_1	4.5	6.8	8.2	6.5	6.5	6.5
K_2	4.5	6.8	8.2	6.5	6.5	6.5
K_3	5.0	6.0	7.8	6.3	6.2	6.2
K_4	3.8	6.2	8.0	6.2	5.8	6.0
		(± 0.31)				(± 0.18)
P_1	4.6	6.2	8.2			6.4
P_2	4.2	6.6	7.9			6.2
Mean	4.4	6.4	8.1			6.3
		(± 0.22)				

were adversely affected by levels of both P and K. So pronounced was the effect of P that some records of the dry weight of leaves and stems were not taken.

(b) FeV experiment. Fresh weight of plants, leaves, and stems and dry weight of plants and leaves showed a positive linear effect of iron level which tended to decrease as iron level increased. Increase in K level decreased the fresh weights of whole plants, leaves and stems. No effects of P level were significant.

(c) FeG experiment. In this experiment growth of plants was so uniformly good that iron level showed no effect on fresh or dry weight of whole plants, the leaves or the stems, nor did phosphorus level have any effect. In contrast the effect of increased potassium level was to depress all these values, though the decreases are small.

Chemical analysis of leaves

A. Phosphorus. The percentage phosphorus in the ash of

TABLE 4

Fe and P in ash of mustard plant leaves. Mean values

Treatment	% Fe						% P					
	Fe_1	Fe_2	Fe_3	P_1	P_2	Mean	Fe_1	Fe_2	Fe_3	P_1	P_2	Mean
$FeCl_3$ experiment												
	(± 0.0037)					(± 0.0021)	(± 0.238)					(± 0.137)
P_1K_1	0.038	0.030	0.042			0.037	3.00	2.79	2.44			2.74
P_1K_2	0.026	0.030	0.032			0.030	3.14	2.86	2.10			2.70
P_1K_3	0.020	0.018	0.034			0.024	3.22	2.88	2.74			2.95
P_1K_4	0.014	0.015	0.027			0.019	2.36	2.94	2.42			2.57
P_2K_1	0.018	0.032	0.030			0.027	2.74	2.90	3.60			3.08
P_2K_2	0.021	0.052	0.020			0.032	4.44	5.45	4.70			4.86
Mean	0.023	0.030	0.031			0.028	3.15	3.30	3.00			3.15
	(± 0.0015)						(± 0.097)					
FeV experiment												
	(± 0.0043)			(± 0.0035)		(± 0.0025)	(± 0.126)			(± 0.103)		(± 0.073)
K_1	0.031	0.040	0.062	0.045	0.044	0.044	2.96	2.98	2.87	2.68	3.19	2.94
K_2	0.030	0.059	0.066	0.042	0.061	0.052	2.98	2.72	2.48	2.66	2.80	2.73
K_3	0.024	0.048	0.062	0.044	0.045	0.045	3.12	2.51	2.56	2.68	2.78	2.73
K_4	0.022	0.040	0.057	0.039	0.041	0.040	3.12	2.73	2.71	2.84	2.87	2.85
	(± 0.0031)					(± 0.0018)	(± 0.089)					(± 0.051)
P_1	0.025	0.043	0.060			0.043	2.90	2.60	2.64			2.72
P_2	0.029	0.050	0.064			0.048	3.18	2.87	2.67			2.91
Mean	0.027	0.047	0.062			0.045	3.04	2.74	2.66			2.81
	(± 0.0022)						(± 0.063)					
FeG experiment												
	(± 0.0059)			(± 0.0048)		(± 0.0034)	(± 0.114)			(± 0.093)		(± 0.066)
K_1	0.061	0.073	0.098	0.074	0.080	0.077	2.69	2.92	3.23	2.82	3.07	2.95
K_2	0.052	0.062	0.082	0.068	0.063	0.065	2.78	2.70	2.74	2.74	2.74	2.74
K_3	0.048	0.063	0.074	0.062	0.061	0.062	2.77	2.90	2.97	2.83	2.94	2.88
K_4	0.058	0.058	0.071	0.060	0.065	0.062	2.92	3.03	2.93	2.84	3.08	2.96
	(± 0.0042)					(± 0.0024)	(± 0.081)					(± 0.046)
P_1	0.056	0.062	0.080			0.066	2.66	2.89	2.88			2.81
P_2	0.054	0.066	0.082			0.067	2.92	2.89	3.06			2.96
Mean	0.055	0.064	0.081			0.067	2.79	2.89	2.97			2.88
	(± 0.003)						(± 0.057)					

TABLE 5

K and Ca in ash of mustard plant leaves. Mean values

Treatment	% K						% Ca					
	Fe_1	Fe_2	Fe_3	P_1	P_2	Mean	Fe_1	Fe_2	Fe_3	P_1	P_2	Mean
$FeCl_3$ experiment												
	(± 1.69)					(± 0.98)	(± 1.26)					(± 0.73)
P_1K_1	13.6	12.5	12.2			12.8	21.0	23.1	24.1			22.8
P_1K_2	20.3	18.7	14.7			17.9	16.3	16.4	20.7			17.8
P_1K_3	22.4	23.5	21.8			22.6	12.5	13.2	16.1			14.0
P_1K_4	33.1	31.9	34.6			33.2	7.7	6.9	9.7			8.1
P_2K_1	13.7	12.9	14.9			13.8	19.8	27.0	20.1			22.3
P_2K_2	36.9	37.8	37.0			37.2	3.2	3.4	2.7			3.1
Mean	23.3	22.9	22.5			22.9	13.4	15.0	15.6			14.7
	(± 0.68)						(± 0.51)					
FeV experiment												
	(± 1.22)			(± 1.00)		(± 0.71)	(± 0.60)			(± 0.49)		(± 0.35)
K_1	16.2	16.3	17.1	16.2	17.0	16.6	20.4	21.8	22.0	22.0	20.8	21.4
K_2	20.6	20.8	21.0	20.7	21.0	20.8	17.2	20.0	19.2	18.7	18.9	18.8
K_3	25.0	20.5	23.7	24.7	21.4	23.0	13.4	19.1	18.2	16.6	17.2	16.9
K_4	32.3	30.7	29.8	31.2	30.6	30.9	7.8	11.2	11.5	10.1	10.3	10.2
	(± 0.86)					(± 0.50)	(± 0.42)					(± 0.24)
P_1	24.1	21.9	23.6			23.2	14.6	18.5	17.5			16.9
P_2	23.1	22.3	22.2			22.5	14.8	17.6	18.0			16.8
Mean	23.5	22.1	22.9			22.8	14.7	18.1	17.7			16.8
	(± 0.61)						(± 0.30)					
FeG experiment												
	(± 1.11)			(± 0.91)		(± 0.64)	(± 1.38)			(± 1.13)		(± 0.80)
K_1	8.6	9.1	9.4	9.1	9.0	9.1	25.8	23.2	21.3	24.1	22.7	23.4
K_2	15.3	16.8	17.3	15.9	17.0	16.4	20.2	21.0	19.5	21.1	19.3	20.2
K_3	15.3	20.7	19.5	19.0	18.0	18.5	13.8	17.1	17.2	16.3	15.7	16.0
K_4	24.6	23.6	25.8	24.1	25.3	24.7	14.0	13.3	12.7	13.1	13.6	13.3
	(± 1.79)					(± 0.45)	(± 0.97)					(± 0.56)
P_1	15.8	17.6	17.7			17.0	18.5	19.0	18.5			18.6
P_2	16.1	17.5	18.4			17.3	18.4	18.3	16.8			17.8
Mean	16.0	17.6	18.0			17.2	18.4	18.6	17.6			18.2
	(± 0.56)						(± 0.69)					

leaves of mustard showed negative linear effect with iron level when iron was supplied as $FeCl_3$. With FeV, phosphorus was only significantly higher at the lowest Fe level whereas with FeG there was no evident response to iron. Potassium had no effect of any consequence on P content in any of the experiments, (Table 4).

B. Iron. Due to the poor growth in the $FeCl_3$ experiment, only at the higher iron level was there any significant increase of iron

in the leaf ash, whereas in the FeV and FeG experiments, positive linear effects were found.

Potassium level showed a negative linear relationship to iron content in the $FeCl_3$ experiment but in the FeG experiment the iron content of the leaves was only significantly greater at the K_1 level than at K_2, K_3 and K_4. Phosphorus had no effect on iron content in the FeV and FeG experiments and insufficient

TABLE 6

Magnesium in ash of mustard plant leaves. Mean values

Treatment level	% Mg					
	Fe_1	Fe_2	Fe_3	P_1	P_2	Mean
$FeCl_3$ experiment						
		(± 0.372)				(± 0.215)
P_1K_1	2.30	2.46	2.16			2.31
P_1K_2	2.37	2.26	2.14			2.26
P_1K_3	2.25	2.48	2.22			2.32
P_1K_4	2.86	2.70	3.14			2.90
P_2K_1	2.07	2.45	2.83			2,45
P_2K_2	2.28	2.15	2.10			2.17
Mean	2.35	2.42	2.43			2.40
		(± 0.152)				
FeV experiment						
		(± 0.118)		(± 0.097)		(± 0.068)
K_1	2.10	2.05	1.75	2.20	1.73	1.97
K_2	2.25	2.05	1.92	2.22	1.93	2.08
K_3	2.40	2.55	2.20	2.55	2.22	2.38
K_4	2.92	2.98	2.95	3.15	2.75	2.95
		(± 0.084)				(± 0.048)
P_1	2.54	2.64	2.41			2.53
P_2	2.30	2.18	2.00			2.16
Mean	2.42	2.41	2.21			2.34
		(± 0.059)				
FeG experiment						
		(± 0.153)		(± 0.125)		(± 0.088)
K_1	2.25	2.18	2.22	2.21	2.22	2.22
K_2	2.15	1.86	1.73	2.10	1.72	1.91
K_3	2.35	2.41	2.43	2.46	2.33	2.40
K_4	3.50	3.42	3.45	3.30	3.62	3.46
		(± 0.108)				(± 0.062)
P_1	2.64	2.44	2.47			2.52
P_2	2.48	2.50	2.45			2.47
Mean	2.56	2.47	2.46			2.50
		(± 0.077)				

measurements were obtained for the $FeCl_3$ experiment due to the poor survival of the plants. (Table 4).

C. Potassium and calcium. Apart from the expected increase in potassium of the leaf with increased nutrient K level and a small increase in K content of the leaf at the higher Fe levels in the FeG experiment, other treatments had no significant effect. Increase of K depressed the level of Ca in the leaf in all experiments.

In the $FeCl_3$ experiment, calcium content of the leaf ash showed

TABLE 7

P/Fe and K/Ca ratios of ash of mustard plant leaves. Mean values

Treatment	P/Fe						K/Ca					
	Fe_1	Fe_2	Fe_3	P_1	P_2	Mean	Fe_1	Fe_2	Fe_3	P_1	P_2	Mean
$FeCl_3$ experiment												
	(± 14.1)					(± 8.1)	(± 0.428)					(± 0.247)
P_1K_1	84	93	58			78	0.66	0.54	0.50			0.57
P_1K_2	125	105	67			99	1.28	1.14	0.72			1.04
P_1K_3	178	156	86			140	1.82	1.78	1.36			1.65
P_1K_4	165	196	98			153	4.31	4.83	3.64			4.26
P_2K_1	160	95	120			125	0.70	0.48	0.74			0.64
P_2K_2	219	112	230			187	11.74	10.67	13.48			11.96
Mean	155	126	110			130	3.42	3.24	3.41			3.35
	(± 5.7						(± 0.175)					
FeV experiment												
	(± 12.9)			(± 10.5)		(± 7.5)	(± 0.113)			(± 0.092)		(± 0.065)
K_1	98	77	46	66	81	74	0.80	0.76	0.78	0.74	0.82	0.78
K_2	109	48	40	73	58	66	1.20	1.05	1.10	1.11	1.12	1.12
K_3	149	52	41	79	82	81	1.92	1.08	1.31	1.56	1.31	1.44
K_4	138	68	48	83	86	84	4.14	2.74	2.61	3.20	3.12	3.16
	(± 9.1)					(± 5.3)	(± 0.08)					(± 0.046)
P_1	121	61	44			75	2.06	1.36	1.55			1.65
P_2	126	62	43			77	1.97	1.46	1.35			1.59
Mean	123	62	44			76	2.02	1.41	1.45			1.62
	(± 6.5)						(± 0.056)					
FeG experiment												
	(± 3.8)			(± 3.1)		(± 2.2)	(± 0.125)			(± 0.102)		(± 0.072)
K_1	48	40	33	39	41	40	0.34	0.40	0.58	0.39	0.50	0.44
K_2	53	44	36	45	44	44	0.76	0.81	0.89	0.75	0.89	0.82
K_3	58	52	40	50	50	50	1.15	1.41	1.14	1.34	1.13	1.24
K_4	45	52	41	44	48	46	1.78	1.78	2.06	1.86	1.89	1.88
	(± 2.7)					(± 1.6)	(± 0.088)					(± 0.051)
P_1	46	50	37			44	1.01	1.15	1.09			1.08
P_2	56	44	38			46	1.00	1.05	1.25			1.10
Mean	51	47	37			45	1.01	1.10	1.17			1.09
	(± 1.9)						(± 0.062)					

a positive linear increase with iron level, whereas in the FeV experiment the iron effect was only marked between the Fe_1 and Fe_2 levels and in the FeG experiment, iron level had no significant effect on calcium (Table 5).

D. Sodium and magnesium. No consistent effect on sodium content of the leaf was found in any of the three experiments. Magnesium content increased with K level in all experiments (probably because Ca decreased rapidly) and was significantly greater at the P_1 level in the FeV experiment, but there was no effect of P for the FeG experiment and insufficient measurements were obtained for the $FeCl_3$ experiment (Table 6).

E. Phosphorus/iron ratio. In the $FeCl_3$ experiment this ratio was greater in the leaves of plants grown at the Fe_1 and Fe_2 levels than plants at the Fe_3 level, while in the FeV experiment the ratio was greater at the Fe_1 level than at the Fe_2 and Fe_3 level. In the FeG experiment the ratio showed a negative linear effect with iron level. Increase in K level increased the ratio in the $FeCl_3$ experiment but had no significant linear effect in either the FeV or FeG experiments. The few results available for the $FeCl_3$ experiments indicate an increase in this ratio to unphysiological dimensions with change in P level, but no significant effect of P (Table 7), was obtained in the FeV and FeG experiments.

F. Potassium/calcium ratio. Increase in iron level decreased the K/Ca ratio in the leaves of the $FeCl_3$ plants between the Fe_2 and Fe_3 levels while a significant difference for the FeV experiment was only found between the Fe_1 level and the Fe_2 and Fe_3 levels. No significant effect of iron was found for the FeG experiment. Phosphorus had no effect on the K/Ca ratio, which showed the expected positive linear response to K level in all experiments (Table 7).

DISCUSSION

Growth, colour, and mineral composition of the mustard plants in these experiments were profoundly affected by the form of iron supplied. As Biddulph and Woodbridge [4] have shown, ferric iron in a nutrient solution containing phosphate is very rapidly precipitated and becomes unavailable to the plants. When iron is chelated with a compound, such as APCA (G), which results in a chelate with a very high stability constant, it is unaffected by

the presence of anions or cations which would otherwise decrease its ionic solubility. The EDTA chelate (FeV) when supplied at very low concentration is however affected by the large amount of calcium in the solution since EDTA forms a chelate with calcium with a relatively high stability constant.

Height of the plants at harvest showed these effects very clearly, increase in iron in the $FeCl_3$ experiment having but a small effect, with the mean value for Fe_3 of 8.6 cm. With FeV there is a marked increase in height with each iron increment with a mean at Fe_3 of 18.2 cm whereas with FeG at even the lowest iron level the plants were 29.5 cm high.

Colour of the leaves showed a very similar trend to height, the mean colour rating for the $FeCl_3$ experiment being 2.7 with little influence of iron level. With FeV there again was an increase in greenness with the mean rating about 4.9 which is only slightly greater than the colour mean for the lowest Fe level in the FeG experiment. Similar effects were obtained for fresh-weight and dry-weight determinations which are not presented.

The mineral composition of the leaves, however, was not much affected by the form of iron. Although the mean iron content of the leaf ash increases through the series, $FeCl_3$, FeV, and FeG, the difference is not great and could be due to the increased solubility of iron in the nutrient solution. A similar effect can be seen for Ca and a possible inverse effect for K but the effects are small.

Mineral composition of the leaf is however, dependent to a great extent upon the levels of iron and other elements in the nutrient solution in all experiments. Apart from the expected increase of a particular element in the ash with increase in the nutrient solution, certain interactions are evident. Thus in the $FeCl_3$ experiment, the percentages of P, Fe, K, and Ca in the leaf ash are all interdependent, Fe being adversely affected by K level, and P being adversely affected by Fe level, while Ca shows an increase with increase of iron level. These effects are less marked in the FeV experiment and are not evident in the FeG experiment.

When the P/Fe and K/Ca ratios are considered, increase in K level of the nutrient medium has the effect of increasing the P/Fe ratio in the $FeCl_3$ experiment and increase of Fe decreases the K/Ca ratio in $FeCl_3$ and FeV experiments. Although these effects are

not significant in the FeG experiment, it should be noted that in the FeG experiment, growth was adversely affected by increase in K level. It is probable that sampling and analytical procedures are too gross to show this relationship where iron supply to the leaf is adequate or nearly so.

The fact that P/Fe and K/Ca show the same trend in the leaves of plants lacking in iron implies an antagonism between Fe and K which is apparently contrary to the work of Hewitt and Bolle-Jones [20] and Bolle-Jones [5] who have postulated a synergistic effect between iron and potassium in the potato plant. Inspection of Bolle-Jones' data however, show that all his findings are consistent with the findings in this study. The synergistic effect between potassium and iron recorded by him can be ascribed to the enhanced translocation of potassium from older to younger leaves at higher iron levels, which would occur in the massive tissues of the potato stems and leaves.

Synergism between iron and calcium has not been studied to any great extent. The observations of Loehwing [24] are in agreement with the present study. The effect of excess calcium in a soil is generally associated with high pH and consequent unavailability of iron (DeKock [10]). Plants growing on such calcareous soils generally contain less calcium (Bennett [3], Lindner and Harley [23]) because iron absorption is prevented. We have found that when FeV was painted on the leaves of iron-deficient mustard plants, the calcium content of the leaf ash increased (Table 8), while the phosphorus content decreased.

TABLE 8

Ash analyses of iron-deficient mustard leaves compared with similar leaves to which FeV had been applied

Treatment	% P	% Fe	% K	% Ca	% Na	% Mg	P/Fe	K/Ca	Symptoms
Control . .	4.27	0.021	21.0	9.8	3.4	2.3	203.3	2.14	chlorotic
FeV painted	3.43	0.070	22.0	12.3	3.4	2.1	49.0	1.79	green

From these studies it is clear that the metabolism of iron and phosphorus are closely connected. There is considerable evidence for the view that iron is attached to phosphoproteins in both animal (Fischer and Hultsch [18]; Bass, Bernick, and Saltman [2]) and plant cells (Noack and Liebich [28]; Possingham and Brown [31]; DeKock [11]). If such bound iron is in the ferric

state (Fischer and Hultsch [18]; Liebich [22]) and iron not so bound in the ferrous state then the P/Fe ratio becomes a measure of the ferric–ferrous balance in the cell.

Brown [6] has recently shown that the synthesis of haem is dependent upon the presence of ferrous iron, the synthesis not proceeding if ferrous iron was present in low or excessive amounts. Chlorophyll synthesis has furthermore been shown to have an intimate correlation with leaf iron and with haem content of the leaf (Sironval [32]). The observations of Oserkowsky [30] on the relation between chlorophyll content of the leaf and iron extractable with normal HCl (active iron) and the additional study of Jacobson [21] support the view that the P/Fe ratio and amount of ferrous iron in the leaf are inversely related.

Moreover the enzyme aconitase has been shown to be dependent upon ferrous iron for activity (Dickman and Cloutier [15]). Its activity may therefore depend upon the iron status of the leaf as measured by the P/Fe ratio. Experiments bearing on this point will be described in a later communication.

The major acids common to plant leaves are, as far as we are aware, citric, malic, and oxalic acids. That there is a balance between citric acid on the one hand and malic plus oxalic on the other has been pointed out elsewhere as also the dependence of this balance on the P/Fe and K/Ca ratios (DeKock and Morrison [13]). It is quite possible that the relation between P/Fe and K/Ca is brought about by the balance of citric and malic (or oxalic) acids which, to a certain extent is dependent upon aconitase and ferrous iron [34].

Similar relationships between P/Fe and K/Ca (and organic acids) are observed in plants suffering from genetical chlorosis or virus attack (Church [7 8]; DeKock and Hall [12] and unpublished) and also in the process of ageing (Colin and Grandsire [9]; DeKock *et al.* in preparation).

It would appear therefore that these ratios are fundamental determinants in metabolism of cells and knowledge gained from a study of the variation of them makes possible interpretations of certain unexplained physiological observations such as stimulation of respiration by an inhibitor (MacDonald and DeKock [26]) or the effect of oxygen tension upon organic acid metabolism (Barker and Mapson [1]; Turner and Quartley [33]).

Recent considerations of the action of plant hormones such as, indolylacetic acid have suggested that they act as chelating agents (Heath and Clark [19]). From a study of growth of maize plants in the presence of organic phosphate which was not supposed to precipitate ionic iron from the nutrient solution, Majumder and Dunn [27] proposed that EDTA acted as a growth substance. The above results and other studies (DeKock [10]) however, clearly indicate that the greater the stability of the iron chelate, the better the growth of the plants. It may well be that certain hormones are chelating agents, but it would be pointless to maintain the converse that all chelating agents are growth hormones.

SUMMARY

Mustard plants were grown in nutrient solutions containing 3 levels of Fe, 4 ratios of K/Ca, and 2 ratios of P/N, the iron in each experiment being applied as the chloride, the ethylene diamine tetra-acetic acid or the ethylene N,N′–*bis* (2–*o*–hydroxyphenyl) glycine chelate. It was found that increases in the iron level decrease the values of both P/Fe and K/Ca as estimated on the leaf ash when iron was an element limiting the growth of the plants.

Received May 22, 1959

REFERENCES

1 Barker, J. and Mapson, L. W., Studies in respiratory and carbohydrate metabolism of plant tissues. Proc. Roy. Soc. London B. **143**, 523–549 (1955).
2 Bass, R., Bernick, S. and Saltman, P., The nucleus in the accumulation of iron by liver cell suspensions. Exptl. Cell Research **13**, 395–397 (1957).
3 Bennett, J. P., Iron in leaves. Soil Sci. **60**, 91–105 (1945).
4 Biddulph, O. and Woodbridge, C. G., The uptake of phosphorus by bean plants with particular reference to the effects of iron. Plant Physiol. **27**, 431 (1952).
5 Bolle-Jones, E. W., The inter-relationships of iron and potassium in the potato plant. Plant and Soil **6**, 129–173 (1955).
6 Brown, E. G., Evidence for the involvement of ferrous iron in the biosynthesis of γ-aminolaevulic acid by chicken erythrocyte preparations. Nature **182**, 131–135 (1958).
7 Church, A. H., A chemical study of vegetable albinism. J. Chem. Soc. **35**, 33–41 (1879).
8 Church, A. H., A chemical study of vegetable albinism, Part III. Experiments with *Quercus rubra*. J. Chem. Soc. **49**, 839–843 (1886).
9 Colin, H. and Grandsire, A., Minéralisation des feuilles vertes et des feuilles chlorotiques. Compt. Rend **181**, 133–135 (1925).
10 DeKock, P. C., The iron nutrition of plants at high pH. Soil Sci. **79**, 167–175 (1955).
11 DeKock, P. C., Heavy metal toxicity and iron chlorosis. Ann. Botany (London) N.S. **20**, 133–141 (1956).

12 DeKock, P. C. and Hall, A., The phosphorus-iron ratio in relation to genetical chlorosis. Plant Physiol. **30**, 293 (1955).
13 DeKock, P. C. and Morrison, R. I., The metabolism of chlorotic leaves 1. Amino acids 2. Organic acids. Biochem. J. **70**, 266–277 (1958).
14 DeKock, P. C. and Strmecki, E. L., An investigation into the fertilizing effect of a lignite. Physiol. Plantarum **7**, 503–512 (1954).
15 Dickman, S. R. and Cloutier, A.-A., Factors affecting the activity of aconitase. J. Biol. Chem. **188**, 379–388 (1951).
16 Ehrenberg, P., Das Kalk-Kali Gesetz. Landwirtsch Jahrb. **54**, 1–130 (1920).
17 Ehrenberg, P., Zusammenfassende Betrachtungen zur Eisenversorgung von Kulturpflanzen. Z. Pflanzenernähr. Düng. u. Bodenk. **45**, 1–83 (1936).
18 Fischer, F. and Hultzsch, K., Uber die Bindung des Eisesn in Eiweisstoffen. Biochem. Z. **299**, 104–122 (1938).
19 Heath, O. V. S. and Clark, J. E., Chelating agents as growth substances. Nature **178**, 600–601 (1956).
20 Hewitt, E. J. and Bolle-Jones, E. W., Studies on the iron deficiency of crops and preliminary experiments on the inter-relationships of iron and potassium in the potato plant. J. Hort. Sci. **28**, 185 (1953).
21 Jacobson, L., Iron in the leaves and chloroplasts of some plants in relation to their chlorophyll content. Plant Physiol. **20**, 233 (1945).
22 Liebich, H., Quantitativ-Chemische Untersuchungen über das Eisen in den Chloroplasten und übrigen Zellbestandteilen von *Spinacea oleracea*. Z. Botan. **37**, 129–157 (1941).
23 Lindner, R. C. and Harley, C. P., Nutrient inter-relationships of lime induced chlorosis. Plant Physiol. **19**, 420–444 (1944).
24 Loehwing, W. F., Calcium, Potassium and Iron balance in certain crop plants in relation to their metabolism. Plant Physiol. **3**, 261–275 (1928).
25 Lundegårdh, H., Leaf Analysis (translated by R. L. Mitchell). London (1951).
26 MacDonald, I. R. and DeKock, P. C., The stimulation of leaf respiration by respiratory inhibitors. Physiol. Plantarum **11**, 464–477 (1958).
27 Majumder, S. K. and Dunn, S., Effect of metal chelates on growth in solution cultures. Plant Physiol. **33**, 166–169 (1958).
28 Noack, K. and Liebich, H., Die Eisengarnitur der Chloroplasten vom Spinat. Naturwiss., 302 (1940).
29 Olsen, C., Iron absorption and chlorosis in green plants. Compt. rend. trav. lab. Carlsberg Ser. chim. **21**, 15–52 (1935).
30 Oserkowsky, J., Quantitative relation between chlorophyll and iron in green and chlorotic pear leaves. Plant Physiol. **8**, 449–468 (1933).
31 Possingham, K. and Brown, R., The nuclear incorporation of iron and its significance in growth. J. Exptl. Botany **9**, 273–284 (1958).
32 Sironval, C., Day-length and haematin compounds in plants. Nature **182**, 1170–1171 (1958).
33 Turner, E. R. and Quartley, C. E., Studies in the respiration and carbohydrate metabolism of plant tissues. VIII. An inhibition of respiration in peas induced by "oxygen poisoning". J. Exptl. Botany **7**, 362–371 (1956).
34 Bacon, J. S. D., DeKock, P. C., and Palmer, M. G., Aconitase levels in the leaves of iron-deficient mustard plants. Biochem. J. **73**, 41P (1959).

Plant and Soil XII, no 2 March 1960

COMPETITION BETWEEN SOIL BACTERIA AND FUSARIUM *

by K. C. MARSHALL and M. ALEXANDER

Department of Agronomy, Cornell University, Ithaca, New York

INTRODUCTION

The phenomenon of competition in its broadest sense is often regarded as being synonymous with directly antagonistic effects such as antibiosis, staling and parasitism. On a strictly ecological basis, competition is defined as an associative effect in which each population adversely affects the other in the struggle for nutrients, oxygen, space or other common needs, the species taking advantage of a common limiting requirement at a faster rate is the more likely to predominate.

This concept of competition has been generally ignored in interpreting most microbiological interactions or merely mentioned in passing as a possible explanation for inconsistencies observed in studies of antibiotic effects. However, in a study of biochemical mutants of *Neurospora crassa*, Grigg [4] has observed a competitive suppression of prototrophic cells resulting from the removal of sugar from the medium by high concentrations of non-growing auxotrophic cells. Apparent competitive effects between strains of rhizobia also have been reported [7 16], but in no case was any attempt made to determine that competition for a limiting requirement was involved. The suppression of certain staphylococci by heavy concentrations of a viridans streptococcus [1] may likewise be the result of competition.

A number of reports have appeared indicating that competition between micro-organisms may be of some significance in soils.

* The investigation was supported in part by a grant from the United Fruit Company. Agronomy Paper No. 471.

Garrett [3] found that the survival of *Ophiobolus graminis* in wheat straw was limited by the nitrogen content of the soil and suggested that the microflora decomposing the wheat straw immobilized the available soil nitrogen. A similar effect has been reported by Blair [2] who also noted a fungistatic influence of carbon dioxide produced during microbial decomposition of straw. The suppression of *Helminthosporium sativum* in laboratory medium by non-antibiotic-producing bacteria has been attributed to competition for limiting nutrients [5]. Competition also seems to account for other cases of inhibition of fungal pathogens by non-antibiotic-producing fungi [14] and a streptomycete [12]. Skinner [13] has reported the possibility of both competitive and antibiotic suppression of a fungus by a single streptomycete. The possible significance of competition between micro-organisms in soil has been discussed by Thornton and Skinner [15].

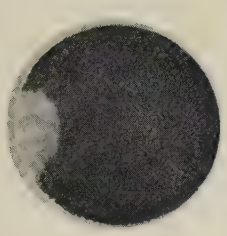
The present study was undertaken to determine whether bacteria are capable of competing with a fungal pathogen in soil, the mechanism of any such action and the basis of competitive ability in these micro-organisms.

METHODS

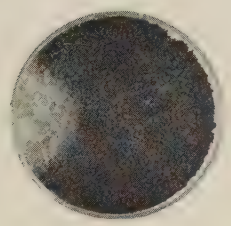
The fungus responsible for vascular wilt of bananas, *Fusarium oxysporum* f. *cubense* (E.F.S.) Sny. and Hans., was used as a test organism. Studies were carried out with stock cultures of *Agrobacterium radiobacter*, *Pseudomonas stutzeri* and isolates from the Honeoye silt loam of New York and two soils from Honduras, the Mopala and San Alejo loams. Honeoye silt loam, pH 7.3, is a well-drained soil developed on a high-lime glacial till.

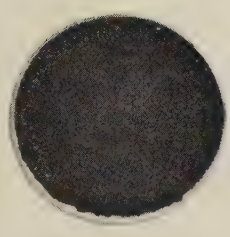
A chemically defined liquid medium, deficient in carbon and nitrogen, was used in attempts to induce competitive effects in mixed cultures. The medium contained 1.5 g glucose, 0.4 g KNO_3, 0.8 g K_2HPO_4, 0.2 g KH_2PO_4, 0.1 g $CaCl_2.2H_2O$, 0.2 g $MgSO_4.7H_2O$ and 0.03 g $FeCl_3.6H_2O$ in 1 liter of distilled water. Where the composition of this medium was altered, the changes are included in the results. Both the fusarium and the bacterium to be tested were inoculated into 250-ml Erlenmeyer flasks containing 50 ml of medium. Controls with the fungus alone were included. After incubation at 30°C for 4 weeks the mycelium was separated by filtering on filter paper, dried at 70°C, and weighed. The competitive ability of 50 isolates from Honeoye soil was determined in nutrient broth by the same procedure.

The method used to measure the growth of the fungus in sterile soil was essentially that described by Park [9]. Twenty grams of air-dry soil were added to each petri plate, moistened to approximately field capacity and

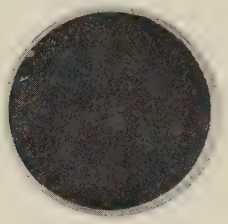


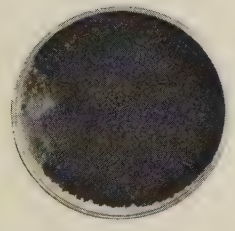
FUSARIUM





FUSARIUM +
AGROBACTERIUM





FUSARIUM +
PSEUDOMONAS

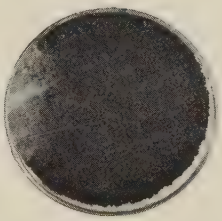


Plate 1. Comparison of the competitive ability of two bacteria in Honeoye silt loam. Incubation period of seven days.

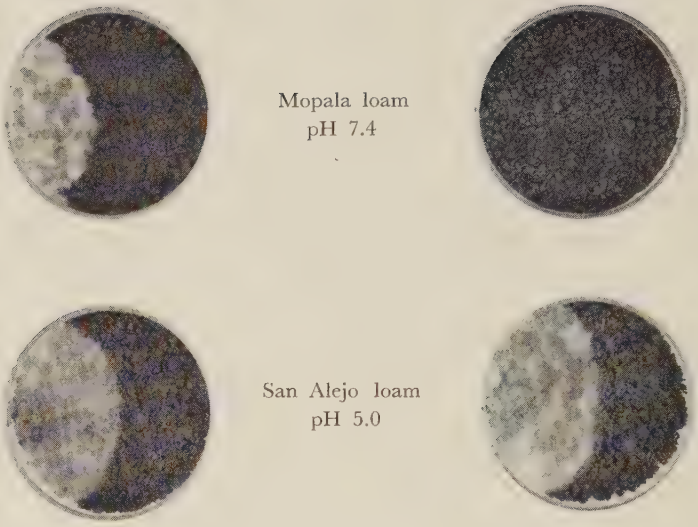


Plate 2. Comparison of the competitive ability of *A. radiobacter* in a neutral and an acid soil. Incubation period of ten days.

autoclaved at 20 lbs. pressure for 90 min. The soil was inoculated with *F. oxysporum* at a point on the side of the plate and, where necessary, inoculated with a bacterium at the same site. The plates were incubated at 30°C, the degree of inhibition of the fungus being determined by measuring radial spread of the mycelium. Competitive organisms were graded on a 1 to 5 basis, 5 representing maximum mycelium development of the fusarium and 1 the greatest suppression in the mixed culture system. In many instances, the sterile soil was amended with 1% glucose or 2.5% KNO_3 that had been sterilized by passage through a Seitz filter.

All the bacteria were tested for antibiotic production against the fusarium by cross-streaking on nutrient agar (Difco). The production of antibiotics in the synthetic medium described and in nutrient broth (Difco) was also examined for many of the isolates.

Growth rates in nutrient broth were determined by following changes in optical density at a wavelength of 625 mμ. From plots of the logarithm of optical density against time, the growth-rate constants, as k values [10], were estimated. Measurement of O_2 uptake in a Warburg microrespirometer was used to determine growth rates of the bacteria in soil. The k values were obtained by plotting logarithm O_2 uptake against time. Each reaction vessel contained 4.0 g sterile air-dry soil, 0.5 ml diluted, washed-cell suspension, 0.5 ml glucose (20 μmole) and 0.2 ml distilled water. To the center well of each vessel was added 0.2 ml of 20% KOH to absorb any CO_2 produced. The gas phase in all these studies was air and the temperature 30°C.

RESULTS

In an initial study of mixed cultures in liquid medium the following mycelium yields were obtained: fusarium alone, 223 mg; fusarium and *P. stutzeri*, 165 mg; fusarium and *A. radiobacter*, 79 mg. Neither bacterium was antibiotically active against *F. oxysporum f. cubense*. As the medium employed was deficient in carbon and nitrogen, the possibility of competition by *A. radiobacter* for one or both of these requirements was examined. The results in Table 1 indicate that the suppression of the fungus under the conditions of study is related to a high C/N ratio, this inhibition being overcome where the ratio is narrow. Apparently the bacterium limits the fungus by immobilizing the nitrogen when the supply of available carbon is high.

Drastic suppression of fusarium by *A. radiobacter* was also observed in sterile, glucose-amended Honeoye soil (Plate 1). Not only was radial growth depressed, but the bulk of mycelium was also reduced. Inhibition of radial spread of the fusarium by *P. stutzeri*

TABLE 1

Influence of varying C/N ratio in liquid medium on inhibition of fusarium by *A. radiobacter*

Treatment		C/N ratio	Fusarium mycelium wt (mg)	
% Glucose	% KNO_3		Fusarium	Fusarium + *A. radiobacter*
3.0	1.6	5.5	274	159
3.0	0.8	11	228	119
3.0	0.2	43	161	92
3.0	0.08	110	101	63
3.0	0.8	11	219	131
1.5	0.8	5.5	102	89
0.75	0.8	2.8	53	70
0.15	0.8	0.5	19	29

was slight, but a reduction in the hyphal mass produced was evident. These effects were also observed, to a lesser extent, in unamended soil.

Many bacteria isolated from the neutral Honeoye and Mopala soils were capable of inhibiting fusarium in the soils of their origin while none of the isolates from the acid San Alejo loam suppressed the fungus in this soil (Table 2). One isolate from the Honeoye

TABLE 2

The abundance of bacteria capable of inhibiting fusarium when tested in the soils of their origin

Grade *	Percentage of bacteria in each grade		
	Honeoye silt loam	Mopala loam	San Alejo loam
1	20	7.5	0
2	18	0	0
3	14	7.5	0
4	0	10	0
5	48	75	100
No. isolates tested	50	40	40
Total no. bacteria per g soil	32×10^6	94×10^5	41×10^4
Soil pH	7.3	7.4	5.0

* Grade 1 signifies maximum inhibition, grade 5 no inhibition.

silt loam could produce an antibiotic active against the fungus in culture media, but it gave only moderate inhibition in soil. Similarly, only one isolate from San Alejo loam produced an antibiotic in culture, but this bacterium did not inhibit the fungus in soil. The

low pH which tends to limit bacterial multiplication probably accounts for the lack of inhibition in San Alejo loam since 4 of a total of 40 isolates did inhibit the fungus in the more favorable Mopala soil. It is noteworthy that one-fourth of the Mopala isolates and half the Honeoye bacteria effectively suppressed the fusarium, yet only one of 90 strains tested produced an antifusarium antibiotic.

The inhibition of fusarium in sterile Honeoye soil by *A. radiobacter* was overcome when the C/N ratio of added glucose and KNO_3 was less than 5 (Table 3), an observation in agreement with the data obtained with liquid media. Thus, it appears that the bacterium suppresses the growth of fusarium by the induction of nitrogen deficiency in the soil resulting from the more rapid utilization and immobilization of the available soil nitrogen.

TABLE 3

Influence of inorganic nitrogen on competition by *A. radiobacter* in sterile Honeoye silt loam. (Incubation period of 13 days)

Nutrient added		C/N ratio of added nutrients	Radial spread of fungus mycelium (cm)	
% Glucose	% KNO_3		Fusarium	Fusarium + *A. radiobacter*
0	0.0	—	2.8	1.2
	0.25	—	2.9	1.0
	0.50	—	3.1	0.8
	2.5	—	2.8	2.9
1.0	0.0	—	5.5	2.1
	0.25	10	5.2	1.9
	0.50	5	5.4	1.9
	2.5	1	5.4	5.0
3.0	0.0	—	5.3	1.8
	0.25	30	5.2	1.8
	0.50	15	5.4	2.0
	2.5	3	5.2	5.2

Eleven soil bacteria giving maximum suppression (grade 1) of fusarium in Honeoye silt loam were tested by the same method. The addition of 2.5% KNO_3 to glucose-amended soil overcame the inhibition created by seven of the organisms tested, indicating that many but not all instances of apparent competition can be explained in terms of rivalry for nitrogen. The mechanism of inhi-

bition by the remaining four bacteria does not appear to be one of competition for limiting nutrients since the inhibition was not reversed by additions of nitrogen or a mixture of other essential elements (0.5% K_2HPO_4, 0.1% $MgSO_4.7H_2O$, 0.1% $CaCl_2$, 0.003% $FeCl_3.6H_2O$, and 0.003% $ZnSO_4.7H_2O$ w/w on a soil basis). Chemical reactions rendering the additions unavailable, however, could not be excluded. None of these bacteria inhibited *F. oxysporum f. cubense* in liquid or solid media. All four are gram-positive, spore-forming rods while the bacteria serving as competitors for nitrogen are all gram-negative, non-spore-forming rods.

The results in Plate 2 clearly demonstrate that *A. radiobacter* was able to compete with *F. oxysporum* successfully in Mopala loam but was unable to do so in the acid San Alejo loam. This effect probably resulted from limited multiplication of the bacterium in the acid soil since competition occurred when the soil was limed to a pH of approximately 6.6.

A relationship between the competitive ability of *A. radiobacter* and soil type in the areas of more acid and alkaline pH (soils 1 to 6) is shown in Table 4. As noted above, this interaction is probably associated with the effect of pH on the multiplication of the bacterium, that is, competition was slight or absent at reactions of 5.7 or below whereas it was most pronounced in Omonita sandy loam, Mopala loam and Palos Blancos clay, all with neutral reaction. Soils 7 to 12 showed no correlation between competitive ability and rate of spread of the vascular wilt fungus. These six are from banana land in Panama and all have an intermediate pH, from 5.9 to 6.8. There is, however, a relationship of physical and chemical characteristics to competitive ability, the greatest suppression consistently occurring in the more sandy soils with low organic matter and nitrogen levels. The addition of nitrogen to all soils where competition was observed overcame the inhibition of fusarium, therefore the mechanism of competitive suppression in these soils, as in Honeoye silt loam, appears to be an induction of nitrogen deficiency by the rapidly growing bacterium. It is tempting to speculate that it is the nitrogen content in Caoba loam, Caoba silty clay and Almirante clay loam which alleviates this effect, but the data do not exclude other explanations.

Competitive ability of the isolates from Honeoye soil was not related to growth rates (k values) of the bacteria in either nutrient

TABLE 4

Length of life, texture and pH as variables influencing competitive ability of *A. radiobacter*

Soil	Length of life *	pH	% Org. matter	% N	% Clay	Radial spread of mycelium (cm) **	
						Fusarium	Fusarium + *A. radiobacter*
1. San Alejo sandy loam	short	5.7	2.1	0.139	10	5.6	3.7
2. San Alejo loam . . .	short	5.0	2.4	0.162	15	5.2	5.0
3. San Alejo clay . . .	short	5.7	4.2	0.236	20	4.8	4.7
4. Omonita sandy loam .	55 + yr	7.4	2.3	0.133	9	3.8	0.5
5. Mopala loam	35 yr	7.4	4.1	0.176	19	4.0	0.4
6. Palos Blancos clay . .	7 + yr	7.5	4.2	0.238	38	4.9	0.2
7. Zapatero fine sandy loam	30 yr	6.8	2.0	0.153	30	5.0	4.0
8. Caoba loam	30 yr	6.4	3.6	0.241	41	5.1	4.9
9. Caoba silty clay . . .	16 yr	6.5	3.4	0.198	46	5.5	5.0
10. Almirante fine sandy loam	short	6.5	0.6	0.061	8	6.1	1.1
11. Almirante silt loam .	short	5.9	2.1	0.133	27	5.3	1.0
12. Almirante clay loam .	short	6.1	3.5	0.138	28	5.9	6.1

* Indicates freedom of the area from the vascular wilt of bananas caused by *F. oxysporum* f. *cubense*.

** Incubation period of 14 days.

broth as determined turbidimetrically or in soil where proliferation was observed by manometric means. The fastest growing strains frequently were not the most effective. Excluding the gram-positive, spore-forming rods, a relationship between competitive capacity and ability to develop in the absence of growth factors was noted in preliminary trials. This observation was confirmed using the nutritional grouping technique of Lochhead and Chase [6], the results in Table 5 indicating that the strongly competitive bacteria have simple nutritional requirements while the less active strains appear to require amino acids or yeast extract in addition to the basal nutrients. No such relationship was found for the *Bacillus* types; this is not unexpected since the mechanism of inhibition by these bacteria was apparently not one of competition for nitrogen.

TABLE 5

Relation of competitive ability to nutritional requirements of soil bacteria						
Grade	Gram reaction	Endospore formation	No. of isolates tested	No. bacteria requiring		
				Basal nutrients only	Basal + amino acids	Basal + yeast extract
1	+	+	4	2	2	0
	—	—	6	6	0	0
2	+	+	4	2	2	0
	—	—	5	3	2	0
3	+	+	3	1	2	0
	—	—	4	0	2	2
5	+	+	13	1	11	1
	—	—	11	1	4	6

DISCUSSION

An association that conforms to the strict definition of competition has been demonstrated between some typical soil bacteria and *F. oxysporum* f. *cubense* in both liquid and soil media. In particular, *A. radiobacter* was found to reduce drastically the growth of the fungus and has been used extensively in the characterization of the nature of this competitive phenomenon. The possibility of the creation of nitrogen deficiency by micro-organisms in soils amended with organic matter [2 3] has been borne out by the results obtained in the present investigation. In such situations, applications of inorganic nitrogen compounds overcame the inhibitory effect on the fusarium.

This demonstration of competition for nitrogen is opposed to Park's [8] contention that the spread of a fungus through soil is limited mainly by antibiotics or staling substances. He claims that the addition of amendments to soil stimulates antibiotic production and that the normal soil solution contains sufficient nutrients for fungus growth, thereby overlooking the possibility of immobilization of available nutrients by other micro-organisms. In the present study, only two of a total of 130 bacteria isolated from three soils showed any antibiotic properties towards *F. oxysporum* f. *cubense*. Further, only one of these inhibited the fungus in glucose amended soil, the degree of inhibition being moderate.

Even this moderate influence may be partly or entirely the result of rivalry for the limited supply of nutrients. Thus, antibiotic production does not appear to be of significance in relation to the limitation of the spread of the fusarium in these soils.

The mechanism of inhibition by the bacillus-type isolates, however, remains obscure. It does not seem to be competition for nutrients at first examination and may be the result of the production of antibiotics, yet this would be surprising since they fail to do so in seemingly ideal conditions in artificial media.

It is obvious that competition by bacteria is unlikely to occur in very acid soils although saprophytic fungi may be of some significance in such habitats. The fact that the organism responsible for the vascular wilt of bananas, *F. oxysporum* f. *cubense*, spreads rapidly in the acid soils of Central America suggests that the native fungi do not actively suppress the pathogen under such conditions. In more favorable pH ranges, competition is of significance in sterile systems, particularly where the initial nitrogen level is low. The wider variety of micro-organisms present in normal field soil would undoubtedly be capable of immobilizing the soil nitrogen more efficiently than would a single species. This effect should be enhanced by the addition of organic matter low in nitrogen to the soil. The postulation of competition for nutrients as a significant means of interaction in non-sterile soils does not involve the restrictions associated with the production of antibiotics such as the requirement for high populations of specific antibiotic-producing micro-organisms or the lack of inactivation of the antibiotics by adsorption, biological degradation or chemical instability [11].

Under the conditions of the present investigations, the competitive ability of soil bacteria is not related to their growth rates. On the other hand, there must be some influence of growth rate upon the capacity of active strains to utilize limiting substrates more rapidly than the fungus. The ability to utilize a simple inorganic nutrient source appears to be the fundamental factor explaining the competitive capacity of the bacteria tested. Such a relationship does not hold for the gram-positive, spore-forming bacteria, but this does not alter the above generalization since none of the bacillus types appear to inhibit fusarium by the mechanism of competition for nutrients.

SUMMARY

The phenomenon of competition has been characterized in liquid medium and sterile soil systems using a variety of soil bacteria and *Fusarium oxysporum* f. *cubense* as test organisms. For most of the bacteria, suppression of the fungus was the result of a biologically induced nitrogen deficiency, this effect being reversed by the addition of excess inorganic nitrogen. High populations of competitors were found in two soils of neutral pH, but no isolates competed in the acid San Alejo loam. *Agrobacterium radiobacter* was able to compete when San Alejo loam was limed to about pH 6.6. Inhibition of the fungus by a number of gram-positive, spore-forming rods could not be accounted for in terms of competition for nutrients or by antibiotic production in artificial media.

The competitive ability of *A. radiobacter* when tested in twelve Central American soils was found to be related to pH in acid and neutral environments but was correlated with texture, organic-matter content and total nitrogen in soils of intermediate pH. In all soils where inhibition occurred, the competitive effect was overcome by additions of inorganic nitrogen. Excluding the group of *Bacillus* spp., the competitive ability of soil bacteria was related to the ability to develop in the absence of amino acids and growth factors but could not be correlated with growth rates of the bacteria in soil or liquid medium.

It is suggested that competition for nutrients is a significant means of ecological control among members of the soil microflora and, together with competitive interactions for space and oxygen, may be the major factors governing the biological control of soil-borne fungi.

ACKNOWLEDGMENTS

The authors are indebted to the Research Department, United Fruit Company for the Central American soil samples and to Dr. R. H. Stover for the fusarium culture.

Received April 24, 1959

REFERENCES

1 Annear, D. I. and Hayes, L., Mixed bacterial growth. I. Enumeration of the viable organisms in mixed populations of a viridans streptococcus and *Staphylococcus albus*. Australian J. Exptl. Biol. Med. Sci. **28**, 183–200 (1950).

2 Blair, I. D., Behaviour of the fungus *Rhizoctonia solani* Kühn in the soil. Ann. Appl. Biol. **30**, 118–127 (1943).

3 Garrett, S. D., Soil conditions and the take-all disease of wheat. V. Further experiments on the survival of *Ophiobolus graminis* in infected wheat stubble buried in the soil. Ann. Appl. Biol. **27**, 199–204 (1940).

4 Grigg, G. W., Competitive suppression and the detection of mutations in microbial populations. Australian J. Biol. Sci. **11**, 69–84 (1958).

5 James, N., Campbell, N. E. R., and Gunner, C., Fundamental studies on a saltant of *Helminthosporium sativum*. Canadian J. Research C **27**, 274–283 (1949).
6 Lochhead, A. G. and Chase, F. E., Qualitative studies on soil microorganisms. V. Nutritional requirements of the predominant bacterial flora. Soil Sci. **55**, 185–195 (1943).
7 Marshall, K. C., Competition between strains of *Rhizobium trifolii* in peat and broth culture. J. Australian Inst. Agr. Sci. **22**, 137–140 (1956).
8 Park, D., On the role of amendments in the biology of fungi in soil. Trans. 6th Intern. Congr. Soil Sci. Paris **III**, 23–28 (1956).
9 Park, D., Behaviour of soil fungi in the presence of bacterial antagonists. Trans. Brit. Mycol. Soc. **40**, 283–291 (1957).
10 Porter, J. R., Bacterial Chemistry and Physiology. John Wiley and Sons, New York (1946).
11 Pramer, D., The persistence and biological effects of antibiotics in soil. Appl. Microbiol. **6**, 221–224 (1958).
12 Siminoff, P. and Gottlieb, D., The production and role of antibiotics in soil. I. The fate of streptomycin. Phytopathology **41**, 420–429 (1951).
13 Skinner, F. A., Inhibition of the growth of fungi by *Streptomyces* spp. in relation to nutrient conditions. J. Gen. Microbiol. **14**, 381–392 (1956).
14 Slykhuis, J. T., Studies on *Fusarium culmorum* blight of crested wheat and Brome grass seedlings. Canadian J. Research C **25**, 155–180 (1947).
15 Thornton, H. G. and Skinner, F. A., The interaction of actinomycetes with other microorganisms in soil. 6th Intern. Congr. Microbiol. Rome, Actinomycetales Symp., 174–190 (1953).
16 Vincent, J. M. and Waters, L. M., The influence of the host on competition amongst clover root-nodule bacteria. J. Gen. Microbiol. **9**, 357–370 (1953).

Plant and Soil XII, no 2 March 1960

NUTRIENT CONTENT OF THE MOIST TROPICAL FOREST OF GHANA

by D. J. GREENLAND * and J. M. L. KOWAL

University College of Ghana

INTRODUCTION

In order to assess the effect of tropical forest vegetation on tropical soils it is necessary to know what quantities of the nutrient elements are taken up and stored in such vegetation. Obviously where vegetative growth is so prodigious, and where the reserves of nutrients in the soil may be depleted by severe leaching, it is to be expected that the vegetation itself may be at least as important as the soil as a storehouse of plant nutrients. Bartholomew, Meyer and Laudelout [2] have determined the nutrients immobilised in forest fallows of up to 18 years of age in the Belgian Congo, and Nye [8] has determined the mean composition of 20-year old forest vegetation in the semi-deciduous tropical forest region of Ghana but the task of measuring the amount and composition of well-developed tropical forest has not previously been attempted. In the present paper the determination of the amount and chemical composition of High Forest in Ghana is described. The nutrient content of the soil supporting the vegetation has also been determined, and the depth and size distributions of the roots.

SITE

The site studied was an area at the University College of Ghana Agricultural Research Station, Kade, at an elevation of 500 ft. This lies within the part of the High Forest Zone described by Taylor [11] as moist semi-deciduous forest, and characterised by the Celtis-Triplochiton association. The local rainfall, about 65 inches per annum, is slightly higher than the average for the moist semi-deciduous forest. A number of rain forest species

* Present address: Waite Institute, Adelaide, Australia.

Fig. 1. Photograph taken at the site at Kade one year after initial clearing (Photo by P. H. Nye).

occur in the vicinity and confirm that the forest here may be at least close to the ecotone between the moist semi-deciduous forest and the rain forest. The actual botanical composition of the vegetation on the site selected together with girth sizes, for all trees of greater than 3 in. girth, is given in Appendix 1. The most common species were *Diospyros spp.* and *Strombosia glaucescens, var. lucida.* There were two very large live trees on the site, *Nauclea diderichii* and *Piptadeniastrum africanum,* both over 10 ft. girth and approximately 200 ft. and 150 ft. high respectively. Natural, undisturbed forest of this type would normally consist of three stories: – 1) the emergents, 150 to 200 ft. high, 2) the upper storey of from 60 to 130 ft. with a closed canopy, and 3) the lower storey with a closed canopy at about 50 ft. The existing forest (Fig. 1) with two emergents to the acre, scattered upper storey and a dense lower storey, is very typical of old secondary forest. The scattered upper storey and dense lower storey indicate interference in the past and point to cultivation having occurred probably 30 to 50 years ago. Local information suggests that it was probably nearer 50 than 30 years previously. In recent years the surrounding area has been worked for exploitable timber, but there was no indication that any had been removed from the actual site. The total basal area of standing timber on the site corresponded to 145 sq. ft. per acre.

The ground flora was mainly herbaceous with some prostrate shrubs, about 1 to 2 ft. high. Typical species were *Leptaspis cochleata, Pollia condensata, Culcasia seandens, Geophila sp., Costus englerianus* and some ferns. The shrub layer grew up to 15 ft. above the ground, and although dense was not so thick as to make it impassable. Typical species were *Pycnocoma macrophylla, Penianthus patulinervis, Baphia nitida, Mabia mualala, Glyphaea brevis, Cnestis ferruginea, Napoleona parviflora* and several young trees of the higher storeys. In parts of the area were some woody vines which had grown up to the tops of trees or groups of trees forming thickets. Prominent species amongst these climbers were *Acacia pennata, Ancistrophyllum spp., Calamus sp.* and others in the families *Conaraceae* and *Apocynaceae.* In the lower corner of the site an area of broken canopy occurred, resulting in a mass growth of thickly interlaced elements including the spiny rattan palm (*Ancistrophyllum spp.*) and the stinging herbaceous climbers *Urera cuneata* and *Fleurya aestuans.*

The soils in the area are very typical of those occurring widely over sedimentary rocks in the forest region of Ghana. They belong to the Bekwai–Nzima–Kokofu–Oda catenary association and form an intergrade between the ochrosols and oxysols, described by the Ghana Soil and Land Use Survey [4]. These Groups are distinguished according to the degree of acidity of the top soil. Those under high rainfall ($>$ 70 in. per annum) with highly acid topsoils (pH 4–4.5) are oxysols, and those under lower rainfall (50–70 in. per annum) where the return of plant nutrients to the topsoil in forest litter counterbalances the effects of leaching, and which therefore have only weakly acid or neutral topsoils, are ochrosols. In the American classification both these soil groups would be termed reddish yellow latosols. The

site chosen was an almost level area of lower mid-slope soils, the slope being approximately 2 : 100 and the aspect westerly. The soils on the upper part of the slope belong to the Nzima series, and grade towards Kokofu down slope. Full profile descriptions indicating the variation found across the site are given in Appendix 2. In general the profile consists of 2 to 3 inches of humous, crumbly silty clay, overlying about 6 inches of pale brown silty to slightly sandy clay, merging into a mottled compact layer of quartz stones and pebbles and ironstone concretions.

PROCEDURE

(a) *Soil sampling and analysis*

The collection of soil samples was carried out prior to any other operations, so that disturbance during the clearing and weighing of the vegetation should not effect the initial soil analyses. The area was laid out in two blocks, each 72 × 40 yd., and each of these blocks divided into 12 plots 6 × 40 yd. Each of these plots was then sampled, 50 cores each of 1½ in. diameter being taken from each plot and bulked to make a composite sample representative of the plot. Twenty-four such composite samples were thus obtained, from each horizon. Each composite sample was analysed and the means of the analyses provided an accurate measure of the soil composition over the site. The samples were taken from the 0–2 in. and 2–12 in. horizons. A few samples were taken from 2–6 in. and 6–12 in. in order that subsurface soil variations could be determined but as these were very small this was not done over the whole site.

The composite samples were air dried on collection, returned to the laboratory where they were oven dried at 105°C, then ground and sieved to pass through a 0.5-mm sieve. The analyses carried out on the dried samples were as follows:

pH – by glass electrode and pH meter, on a 1 : 5 soil suspension in distilled water.

Carbon – by standard Walkley-Black procedure, applying a correction for 77% recovery.

Nitrogen – by the Kjeldahl technique using a potassium sulphate–copper sulphate–selenium catalyst.

Readily available phosphate – by a slightly modified Truog method, the extraction being carried out by shaking 2.0 g of soil with 100 cc of 0.002 *N* sulphuric acid for 1 hour.

Exchangeable cations – by displacement from the soil with 1 *N* ammonium acetate at pH 7 and analysis of the extract for:

Potassium – by Flame photometry,

Calcium plus magnesium, and calcium – by versenate titration, using Eriochrome Black T and Patton & Reder's reagent as indicators.

Magnesium – (as a check) by Titan Yellow, with compensatory solution.

Exchange capacity – by paranitrophenol.

Soil density was determined by weighing the soil by horizons in 6 pits, each $4\frac{1}{2}$ ft. $\times$ 2 ft., distributed randomly in the area immediately adjacent to the site.

(b) *Vegetation determination, sampling and analysis*

The surroundings of the site and the strip between the two blocks were initially cleared to simplify access to the area. The vegetation on the site was then determined in the following categories: roots; stumps; litter; lianes; trash; medium wood; large wood; large dead wood.

Root material included all plant material below ground level, except the underground parts of the larger stumps. These were determined with the stumps, which were winched out of the ground, then cut off at ground level to separate 'stem' and 'root' portions. Litter consisted of all material lying on the surface of the ground and not incorporated with the soil, excluding large, dead wood. Lianes included all climbing plants, complete with their leaf and twig portions. Trash included all leaf material and small wood, up to 2 in. diameter. Medium wood included all wood from 2 in. to 1 ft. diameter, and large wood all wood of greater diameter than this. Large dead wood included all dead wood which had not been included in the litter.

The procedure used for measurement of the vegetation was as follows:

(1) All lianes were removed, the material on each plot being bundled and weighed. It was then extensively subsampled, the subsamples being sealed into polythene bags and returned to the laboratory, where the moisture content was determined by oven-drying at 105°C. The dried material was ground in a Christy-Norris Mill and further subsampled to obtain material for analysis.

(2) 12 areas were marked out, each 5 yd. $\times$ 4 yd., distributed randomly over the site, and litter on each of these areas was collected by hand. There was no difficulty at the time of sampling (mid-dry-season) in distinguishing between soil surface and litter layer, the boundary being perfectly clear. The material was weighed and sampled and returned to the laboratory for analysis as for the lianes.

(3) The whole area was clear felled. All medium wood was cut and corded, according to the plot from which it came. Each cord was weighed, using a 600-lb. Salter weighbridge, and a saw cut made across the complete cord. The sawdust so obtained was sealed in polythene and used for moisture determination and analysis. Twenty-four "composite" samples of small wood were thus obtained. To assess the relative importance of the stem bark and stem wood a duplicate set of sawdust samples were collected from the wood after the bark had been stripped off.

(4) All leaf and twig from the felled material was bundled and weighed, and samples taken for moisture determination and analysis. During felling a considerable addition of leaf and twig to the litter layer occurred. This was measured by repeating the litter determination made previously and determining the fall of leaf and twig by subtraction. This was then added to the quantity of trash which had been determined directly.

(5) The large trees were carefully measured to obtain the volume, and samples removed for dry density determinations. Large dead wood was determined similarly. For analysis sawdust from a cut across the whole tree was used. As it was considered probable that the composition of samples of dead wood of different ages would differ considerably all large specimens were sampled and analysed individually.

(6) Stumps were winched out of the ground on 12 plots randomly selected from the 24, and weighed, the stumps being cut where necessary to make them of manageable size (< 600 lb.) Stem and root portions were weighed separately, the division being made at the point of root branching or for buttressed trees at ground level.

(7) Roots were determined in 14 pits distributed in at random over the area. To ensure that there was no bias in the siting of the pits the sites were selected by a random procedure on a large scale map of the site. Each pit was dug 5 ft. long $\times$ 3 ft. wide $\times$ 4 ft. deep. A strong, sharp-edged steel sheet 3 ft. long and 1 ft. wide was then driven horizontally into the longer sides of the pit at a depth of 1 in. and the roots in the soil above it removed by hand sorting. When all the soil above the plate had been examined it was reinserted at 3 in. depth and the process repeated, and subsequently at depths of 5, 7, 12, 24, 36, and 48 in. and in a few pits where the gravel did not make digging extremely difficult, at 60 and 72 in. This was then repeated on the second longer face of the pit. Large roots were removed whole when found, but marked to indicate their portions by depth. They were then cut, dried, weighed and the weights included with the remainder of the root material from the particular horizon. Root material from each horizon and each face of each pit was weighed separately, but for analysis the material was bulked and analysed according to root sizes, the categories used being roots of diameter less than $\frac{1}{4}$, $\frac{1}{4}$ to $\frac{1}{2}$, $\frac{1}{2}$ to 1, and greater than 1 in. The smaller roots were dried and ground directly in the Christy-Norris Mill, and the larger — after being first reduced to suitable size for grinding by chopping with cutlasses.

Chemical analysis of all dried and ground material was done by the rapid wet-digestion technique of Hutton and Nye [6]. In this method the plant material is digested with concentrated sulphuric acid and selenium. Nitrogen, present in the digest as ammonium, is determined by alkaline distillation, phosphorus by the molybdate method, magnesium by Titan Yellow and potassium by flame photometry. The only modification made to the original method was that calcium was determined by the versenate method.

ERRORS

The errors in the determination of the amounts and composition of the vegetation will first be considered, and then the error in the determination of the soil-nutrient storage. The relationship of the results obtained for the site studied to other areas of tropical forest will be considered subsequently. It is emphasised that the aim of the present study was to make an accurate determination of the distribution of nutrients on one particular site, and it

is the errors in this determination that are now considered, not the 'error' of the difference between the composition of the forest studied and a hypothetical true mean composition of all similar forest vegetation.

(a) *Errors in the measurement and sampling of the vegetation*

The errors involved in the determination of the weights of material were small. Thus *all* woody material on the site, except for very large trees, was weighed. The very large trees were individually measured after felling, and their weights calculated from density measurements on material from the actual tree concerned. The almost perfect cylindrical form of the large tree stems enabled their volumes to be determined with a high degree of accuracy. Leaf and twig and lianes were also weighed for the whole site, and stumps for one half of the site. Litter and roots were estimated from a number of sample areas. The variations between the samples were quite large (cf the standard errors given in Table 1) but as the total quantities of litter and roots represented only a small fraction of the material on the site as a whole the total error introduced is relatively small.

Great care was taken to ensure that each category of vegetation was adequately sampled. When the material from each plot had been weighed large samples, of several kilogrammes each, were extracted by standard techniques for obtaining random samples, and these large samples dried and ground and subsequent subsampling done by quartering of the thoroughly mixed ground material. As stated previously wood was sampled as sawdust from cuts across randomly composed cords. In order to assess the variation between subsamples a number of replicate analyses were carried out. The variations were all found to be within the limits of the analytical methods used.

(b) *Errors in the chemical analysis of the vegetation*

The error of the analytical methods employed for nitrogen, phosphorus and potassium is very unlikely to have exceeded 2 per cent. Magnesium could not be determined as accurately. Hutton and Nye [6] estimate that the error should not exceed 5 per cent, which agrees with the variation found between replicates in the present study. Calcium was estimated by versenate titration. Recovery tests indicated that an accuracy of 2 per cent could be obtained by this method but comparison of replicate samples indicated that in routine analysis the limit of error is probably about 5 per cent. The net effect of the uncertainties in the amount and composition of the vegetation is shown in Table 4. It is seen that the determinations of nutrient immobilisation are not likely to be in error by more than 10 per cent.

(c) *Errors in the measurement of the soil nutrient content*

In assessing the nutrient content of the soil in terms of pounds per acre it is essential not only that the soil be adequately sampled and accurately

analysed, but also that the soil density be accurately known. This was therefore determined on a number of large soil pits, and not by taking small cylindrical cores as is usually done. The results will be considered in detail in a subsequent paper reporting the soil changes taking place on clearing, burning and cultivating. It may be stated here that the standard error between the different pits was for the 0–2 in. horizon 6.8 per cent of the mean, and for the 2–12 in. horizon 5.2 per cent. This error determines the soil nutrient storage error.

On soils similar to that studied here 50 cores per plot of $\frac{1}{20}$ acre should provide a composite sample with a nutrient content within 2 per cent of that of the plot as a whole, with a probability of 1 in a 100. The analytical techniques employed also give results accurate to 2 per cent. It must be remembered however that "Truog" phosphate and, to a less extent, exchangeable-cation content, are somewhat arbitrary measurements. This again will be discussed in more detail when the soil changes are considered further.

The errors to which the uncertainty in the soil-density determination can lead are shown in Table 6. In magnitude they are similar to the errors in the vegetation determination, except for nitrogen, where the error is larger for the soil determination, and phosphorus, where the error is very much larger for the vegetation determination.

RESULTS AND DISCUSSION

The amount of vegetation

The total dry weight of vegetative material on the site (Table 1) was approximately 150 tons, of which 100 tons was stem timber and large dead wood. There are very few data from the tropics against which this may be compared. The data for 18 year old forest fallow [2] indicate about 50 tons per acre of live wood and only about 7 tons of dead wood. This difference is what might be expected considering the difference in the age of the vegetation at the two sites. It is difficult to compare the other components of the vegetation as the categories are not exactly similar. In particular much more woody material was included in the trash and litter of the present study, than was included with leaf and litter in the Yangambi study. Roots are apparently greater at Yangambi in the younger fallow, but in this fallow *Musanga cecropioides* was dominant, and being a stilt-rooted tree little stump material can have been present. Thus the Yangambi 'roots' should be compared with 'roots' plus 'stumps below ground' in the present study. The order of magnitude is then reversed.

For the amount of stem timber in primary forest similar to that at Kade, estimates have been made in the Ivory Coast. The values obtained correspond to 140,000 to 170,000 lb./acre dry weight (Bellouard, quoted by Nye [8]). This agrees excellently with the

TABLE 1

Total weight of vegetation in old (ab. 50 years) secondary forest at Kade, Ghana with data for young secondary forest in a similar environment at Yangambi, Belgian Congo [2]

Site and component of vegetation	Oven-dry wt. lb./acre	Estimated or standard error	Remarks
Kade			
Trash	22,780	± 2,400	Included small wood up to 2 in. diameter.
Lianes	12,900	Nil	Included leaf and twig of lianes.
Litter	2,020	± 610	Sampled mid-dry-season.
Medium wood	57,500	Nil	All wood 2 in. to 1 ft. diameter.
Large wood	97,000	Nil	
Large dead wood	64,100	Nil	
Stumps Above ground	17,500	Nil	
Stumps Below ground	26,200	Nil	
Roots	22,100	± 8,720	
Total	322,100	±11,730	
Yangambi			
Leaves	5,800		Secondary forest, 18 years, dominated by *Musanga cecropioides* [2].
Litter	5,000		
Wood	104,000		
Dead wood	15,500		
Roots	28,000		
Total	158,300		

155,000 lb./acre found at Kade. The only other data with which comparison may be made are unpublished results for primary forest dominated by *Scorodoephleus zenkeri*, quoted by Beirnaert [3]. The amount of medium plus large wood reported is 240,000 lb./acre, but it is not stated whether this refers to green weight, air-dry or oven-dry material. It seems likely that it must refer to green weight. Even so it indicates an exceptionally dense type of forest. Within the zone of moist semi-deciduous forest it is likely that the Kade figures represent a fair average of the amount of vegetation to be found on any one acre of mature forest, although obviously the amount for any particular acre will vary depending on the number

of very large trees present. The basal area, 145 sq. ft., agrees closely with Dawkins' limiting value for tropical high forest [5].

The composition of the vegetation

It is seen from Table 2 that the composition of the vegetation is similar to that of the mixed forest vegetation at Kumasi, Ghana, analysed by Nye [8], but rather richer in nutrients, particularly

TABLE 2

Mean composition of forest vegetation at Kade, together with data from other parts of the world for comparison.

Site and component of vegetation	Percentage composition					Remarks
	N	P	K	Ca	Mg	
Kade, Ghana						
Trash	1.88	0.126	0.76	1.90	0.26	
Lianes	1.18	0.051	0.43	1.94	0.15	
Litter	1.54	0.057	0.45	1.98	0.24	
Medium wood with bark	0.44	0.027	0.255	0.75	0.10	
Medium wood without bark	0.35	0.027	0.22	0.44	0.05	(All figures refer to oven-dry material)
Large wood with bark	0.41	0.025	0.24	0.48	0.09	
Large dead wood	0.32	0.026	0.05	0.73	0.07	
Stumps	0.38	0.040	0.19	0.38	0.07	
Roots	0.86	0.045	0.35	0.59	0.18	
Kumasi, Ghana						
Leaf	2.52	0.136	0.85	1.54	0.48	Means of determinations on 14 mixed deciduous sp. [8]
Litter	1.29	0.054	0.44	1.59	0.31	
Wood with bark	0.32	0.025	0.30	0.31	0.15	
Belgian Congo						
Wood without bark	0.266	0.023	0.200	0.302	0.075	Means of determinations on 67 timber sp. [7]
Wood with bark	0.27	0.30	0.25	0.54	0.090	
Yangambi, Belgian Congo						
Leaf	2.2	0.12	1.24	1.18		18 year old secondary forest dominated by *Musanga cecropioides* [2]
Litter	1.36	0.05	0.145	1.20		
Wood with bark	0.26	0.05	0.27	0.33		
Roots	0.47	0.11	0.64	0.85		
Minnesota, U.S.A.						
Leaf	2.24	0.22	1.09	1.92	0.54	Data from Ref. [1] quoted by Nye [8]
Europe						
Wood with bark (hardwood sp.)		0.019	0.09	0.42		Data from Ref. [10] quoted by Nye [8]

calcium and magnesium, than the 18-year old fallow at Yangambi, dominated by Musanga [2]. This difference is not surprising, as the soil at Yangambi is extremely low in exchangeable calcium and magnesium (Table 6). A similar but smaller difference exists between the composition of the wood at Kade and the mean for 67 different timber species in the Congo [7]. As would be expected the trash, litter, and lianes are of somewhat similar composition and rather richer in nutrients than the remainder of the vegetation. The bark is much richer in calcium and magnesium than the wood, and slightly richer in nitrogen and potassium, but not richer in phosphorus. The finer roots are richer in nutrients than the larger roots (Table 5), the differences being most pronounced with nitrogen and phosphorus. All roots except those greater than 1 in. diameter are richer in nutrients than the stem wood. The roots of the fallow at Yangambi have a higher nutrient content than the mean of the roots at Kade, but this is probably due to the preponderance of finer roots in the younger fallow.

Comparison of the Kade data with the temperate zone data quoted in Table 2 indicates a relatively close similarity in the composition of leaf material, but a higher content of nutrients in the tropical woods than in the temperate-zone hardwoods.

The variations in the composition of medium wood, trash and lianes between the different plots on the site are indicated by the

TABLE 3

Standard error of the mean composition of trash, lianes, and medium wood on the site

Component	Standard error, % of mean					No. of plots analysed
	N	P	K	Ca	Mg	
Trash	4.4	6.0	8.3	5.0	7.0	9
Lianes	4.3	5.1	4.9	4.9	10.7	18
Medium wood with bark .	5.9	8.9	8.2	7.9	13.0	24

standard errors given in Table 3. It is seen that most are below 10 per cent, the majority being close to 5 per cent. A sample of adequate accuracy could therefore be obtained from less than the number of plots sampled in the present study, particularly in respect of trash and lianes, the composition of which varies rather less than that of the wood.

The amounts of nutrients stored in the vegetation

The amounts of nutrients stored in each category of the vegetation and the total nutrient storage are given in Table 4. The uncertainties in the determinations, which arise from the un-

TABLE 4

Nutrient content of forest vegetation at Kade together with data for comparison

Site and component of vegetation	Nutrient content, lb./acre				
	N	P	K	Ca	Mg
Kade					
Trash	428 ± 45	29 ± 3.1	173 ± 18	433 ± 46	59 ± 6.2
Lianes	152 ± 3	7 ± 0.1	56 ± 1.1	250 ± 13	19 ± 1.0
Litter	31 ± 9	1 ± 0.3	9 ± 2.7	40 ± 12	5 ± 1.5
Medium wood with bark	253 ± 5	16 ± 0.3	147 ± 2.9	431 ± 21	58 ± 3.0
Large wood with bark	399 ± 8	24 ± 0.4	231 ± 4.6	466 ± 23	91 ± 4.5
Large dead wood	205 ± 4	17 ± 0.3	32 ± 0.6	468 ± 24	46 ± 2.3
Stumps	166 ± 3	18 ± 0.4	83 ± 1.7	166 ± 8	31 ± 1.5
Roots	191 ± 75	10 ± 3.9	78 ± 30.7	130 ± 51	39 ± 15.4
Total	1,825 ± 152	122 ± 8.8	809 ± 62	2,384 ± 187	348 ± 35.4
Yangambi [2]					
18-year old fallow, Total	625	96	536	733	
Europe [10]					
Conifers at 50 years, Total		66	307	583	
Hardwoods at 50 years, Total		64	278	1,023	
Conifers at 100 years, Total		90	516	966	
Hardwoods at 100 years, Total		110	496	1,938	
Nutrient removal during 50 years of a maize-maize- cassava rotation *	2,400	450	2,500	225	300

* In maize grain and cassava tubers, assuming 2,000 lb. maize grain and 15,000 lb. cassava tubers per acre per crop.

certainty in the determination of the total quantities of trash, litter, and roots and the errors in the chemical analyses, are also shown. The total quantities of nutrients immobilised in the vegetation are much greater than in the young fallow at Yangambi, and also very much greater than the amount immobilised by a

TABLE 4*a*

Percentage of the total nutrient content immobilised in each component of the vegetation at the Site, Kade (Table 4)

Component of vegetation	N	P	K	Ca	Mg
Trash	23.4	23.8	21.3	18.2	17.0
Lianes	8.3	5.7	6.9	10.5	5.5
Litter	1.7	0.8	1.1	1.7	1.4
Medium wood with bark	13.9	13.2	18.1	18.1	17.0
Large wood with bark	21.9	19.7	28.5	19.6	26.1
Large dead wood	11.2	14.0	4.0	19.5	13.2
Stumps	9.1	14.8	10.3	7.0	8.9
Roots	10.5	8.2	9.6	5.4	11.2

hardwood forest of equivalent age in Europe. The quantities are in fact rather larger than found in a temperate hardwood forest of twice the age of the Kade forest. The difference is presumably related to the much longer growing season and more active growth of the tropical forest.

The quantities of nutrients immobilised may also be compared with the nutrient removal effected by 50 years of a maize–maize–cassava rotation. Assuming everything except grain and tubers to be returned to the soil, the calcium removal is only a fraction of the forest content, the magnesium removal about the same as the forest content, the nitrogen removal is slightly greater, but the potassium and phosphorus removals are about three and four times respectively as large as the forest content. The nutrient removal in grain and tubers does not of course represent the only nutrient loss during cultivation. Leaching losses would also be expected to be greater than under the growing forest. There are however very little data at present available to indicate what such losses may be in humid tropical conditions, either under forest or under cultivation.

It is interesting to note that the total nitrogen immobilisation in a forest of not more than 50 years standing was 1,825 lb./acre. The corresponding mean annual nitrogen gain is 36.5 lb./acre. As the soil organic nitrogen also increases under a developing forest vegetation, and as losses of nitrogen by leaching and denitrification will also be occurring during the growth of the forest, it is obvious that the actual fixation rate must be considerably greater than 36.5 lb./acre per annum. The proportion of leguminous species among the trees on the site was only 8.3 per cent of those 3 in. to 1 ft. girth

and 3.8 per cent of those over 1 ft. girth. Thus it seems somewhat unlikely that much of the nitrogen fixation can be attributed to symbiotic fixation.

The proportions of nutrients stored in the readily combustible and less readily combustable parts of the vegetation are important in relation to the nutrient addition to the soil on clearing and burning such forest for cultivation. Large wood, large dead wood, stumps, and roots are the parts of the vegetation usually left unburnt. Roots, however, mostly remain in the soil and their nutrient content is added to the soil during their decomposition. They should therefore be considered part of the combustible material. The larger roots may not decompose during a cropping period, but it is seen from Table 5 that while they constitute a

TABLE 5

Root distribution, composition and nutrient content by size, for 0–48 in. depth, under 50 year old forest at Kade

Mean root diameter, in.	lb./acre dry weight	Nutrient composition, % wt.					Nutrient content, lb./acre				
		N	P	K	Ca	Mg	N	P	K	Ca	Mg
0–¼	4,450	1.37	0.079	0.56	0.88	0.10	61	3.5	25	39	9.0
¼–½	3,850	1.19	0.074	0.58	0.78	0.18	49	2.9	22	30	9.6
½–1	5,210	0.91	0.048	0.48	0.68	0.05	47	2.5	25	35	13.6
> 1	8,590	0.40	0.014	0.07	0.30	0.08	34	1.2	6	26	7.2
Total	22,100						191	10.1	78	130	39.4

large proportion of the total mass of roots, the nutrients they immobilize are only about 20 per cent of the root immobilisation of nutrients. Their slower decomposition is therefore relatively unimportant.

From Table 4*a* it is seen that the nutrients contained in large wood, large dead wood, and stumps are 40 to 50 per cent of the total. Thus only slightly over half of the immobilised nutrients will be immediately released to the soil when forest such as this is cleared and burnt. Even so the immediate additions, equivalent to about 8 cwt. of single superphosphate, 8 cwt. of potassium chloride, over a ton of limestone and about 4 cwt. of magnesium carbonate, are very considerable. The relationship of these additions to the observed soil changes on clearing and burning will be considered in a subsequent paper.

TABLE 6

Analyses of soils under mature forest vegetation
Kade data given with standard errors between plots

Site	Depth, in.	pH	% C	% N	ppm P as "Truog PO_4"	me/100 g exchangeable			me/100 g C.E.C.
						K	Ca	Mg	
Kade	0– 2	5.28 ± 0.08	2.28 ± 0.08	0.198 ± 0.008	8.4 ± 0.56	0.46 ± 0.01	5.52 ± 0.61	1.11 ± 0.12	9.22 ± 0.30
	2–12	4.74 ± 0.10	0.74 ± 0.05	0.101 ± 0.007	2.0 ± 0.29	0.33 ± 0.01	2.32 ± 0.25	0.59 ± 0.04	6.10 ± 0.12

Nutrient content of soil in lb./acre

Site	Depth, in.	pH	C	N	Av. P	Exchangeable			Reference No.
						K	Ca	Mg	
Kade	0–12	4.9	39,000	4,100 ± 260	11.4 ± 1.5	580 ± 31	2,300 ± 130	330 ± 19	
Kumasi	0–12	6.4		6,000	13	220	4,200	450	8
Yangambi	0–12	4.1		2.500	17	300	89	47	2
Liberia	0–12	4.45		3,900	30	450	530	230	9

	Depth, in.	lb./acre	% S.E.
Wt. of oven-dry soil under 50-year old forest at Kade	0– 2	464,000	6.8
	2–12	3,775,000	5.2

The amounts of nutrients stored in the soil supporting the vegetation

The results of the soil analyses are given in Table 6. The standard errors of the means calculated from the analyses for each of the 24 plots are given with the soil-composition data. The quantities of nutrients stored per acre have been calculated from the weight of soil per acre, data for which is also given in Table 6. The errors in the nutrient storage have been calculated from the errors in the soil-density determination.

The analyses are typical of a mature forest latosol, intermediate between the very acid latosols (oxysols) found under rain forest proper and the latosols of only weakly acid or neutral topsoil (ochrosols) found under the drier types of tropical high forest. This can readily be seen if the results of the Kade analyses are compared with the data for the weakly acid ochrosol from Kumasi [8] and the oxysols from Yangambi [2] and Liberia [9], quoted in Table 6. The differences are most apparent in the exchangeable calcium and magnesium contents. The available phosphates and the exchangeable potassium figures depend very much on the reserves of these elements in the soil in forms not subject to leaching. The nitrogen content is considerably less than in drier forest areas, and the humus horizon relatively thin. This is probably associated with a low activity of soil fauna in these more acid soils, but may also be partly due to the compact subsurface horizons of the silty clay soils at Kade.

The relationship of the nutrient contents of soil and vegetation

The ratios of the soil nutrients in the top foot of soil to the nutrients immobilised in the vegetation are given in Table 7. While the total soil nitrogen is larger than the amount of nitrogen in the vegetation, the amounts of available soil phosphorus and exchangeable potassium, calcium and magnesium are less than the vegetation content. Since the exchange complex under the forest

TABLE 7

Ratio of nutrients in soil* to nutrients in vegetation and roots
(*N and C as total; P as available PO_4; K, Ca, and Mg as exchangeable)

Vegetation	N	P	K	Ca	Mg	C
(1) Total vegetation . .	2.6	0.093	0.72	0.96	0.95	
(2) Roots	24.7	1.14	7.4	17.7	8.5	4.0

is more than 50 per cent saturated it is evident that either the exchangeable potassium, calcium and magnesium have been replenished from non-exchangeable sources during the period that the forest has been absorbing nutrients, or alternatively the forest has been drawing extensively on supplies at depths below one foot. Another explanation is that the exchange capacity may have fallen during the development of the forest vegetation, but this is highly improbable. If any change occurs it is likely to be an increase.

It is widely believed that an important function of forest fallows in restoring soil fertility is to withdraw nutrients from the subsoil and store them in the vegetation until such time as the forest is cleared and the nutrients added to the surface soil as ash.

TABLE 8

Root distribution by depth (dry wt., lb./acre)											
Kade					Yangambi *						
Age of vegetation	15 years		50 years		2 years		5 years		8 years		
Depth in.	Wt.	% +	Wt.	% +	Depth in.	Wt.	Depth in.	Wt.	Depth in.	Wt.	% +
0– 1	2,520	15.1	5,520	23.8	0– 4	4,100	0–20	21,500	0–10	13,600	67.6
1– 3	3,270	34.9	5,540	48.7							
3– 5	2,970	52.8	3,550	64.7					10–20	3,570	85.3
5– 7	2,130	65.6	1,940	73.4	4–12	1,820					
7–12	2,310	79.5	2,660	85.5			20–40	1,100			
12–24	1,560	88.9	2,000	94.5	12–24	260			20–30	2,210	96.3
24–36	1,840	100.0	650	97.4					30–40	320	97.9
36–48	—		350	99.0			40–60	390			
48–60	—		150	100.0					40–60	480	100.0
Total	16,601		22,100			6,180		22,990		20,170	

+ % refers to percentage of total weight found in the soil above the lower depth of the horizon for which the figure is given.

* We are indebted to Prof. W. V. Bartholomew for permission to quote these unpublished results.

The data given apparently support that view. Nevertheless there remains the difficulty of reconciling the apparent withdrawal of nutrients from the subsoil with the extremely limited root penetration below 12 in. The data presented in Table 8 show that 85.5 per cent by weight of roots under the 40-year old forest at Kade occur in the top foot of soil. Unfortunately root distribution by weight is not necessarily correlated with feeding activity. Even

so such a great preponderance of roots in the surface may at least be taken as an indication that most nutrients are adsorbed from the surface horizons. Furthermore the much better aeration of the surface horizons would make it probable that the surface roots were more active feeders than the subsoil roots. If then the 40-year old forest is feeding predominantly in the surface soil, how has it obtained its large content of nutrients? One possibility is that the relatively few roots which penetrate the subsoil are particularly voracious feeders. Two other possibilities at least merit further investigation. One is that the roots penetrate the subsoil more extensively during earlier stages of development than in the later stages. The data for the root distribution under forest of about 15 years at Kade, in soil very closely similar to that of the 40-year old fallow, shows only 79.5 per cent of the roots in the top 12 in. of soil, and in an 8-year old fallow at Yangambi Bartholomew * found only 67.6 per cent in the top 10 in.

The other possibility is that important quantities of nutrients are released from non-exchangeable sources in the surface soils. Tropical latosols are generally considered to certain very small reserves of non-exchangeable cations, but further experimental examination of this fact is desirable. It is possible that a forest fallow functions by making non-exchangeable nutrients available to a succeeding crop, rather than by making subsoil nutrients available.

It is often suggested by agriculturists that the decaying roots of former forest vegetation provide an important source of plant nutrients, which become available over a number of years after the forest is cleared.It is also suggested that they may provide a source of organic material to replenish that which is lost during cropping. The data in Table 7 indicate that it is unlikely that their nutrient content is of much importance, the initial soil content of available nutrients being several times greater than that in the roots. Furthermore the nutrients added to the soil on clearing the vegetation will make the relative importance of the nutrients in the roots even less. The amount of carbon they contain is about 25 per cent of that in the soil organic matter. They may therefore be of some importance as a source of organic material, although much of the carbon in the roots is likely to be oxidised during the processes of

* Private communication.

humification. The effect of the roots during subsequent cultivation is being investigated by cultivating some of the 24 plots at Kade with and without removal of roots and stumps from the surface horizons. It is of course possible that the old roots have a beneficial effect during cultivation by providing channels into the subsurface soil for the movement of air and water.

SUMMARY

The total weight of vegetation on an area of just over 1 acre of old secondary forest in the moist forest zone of Ghana has been determined, and found to be equivalent to roughly 150 tons per acre dry weight. The nutrient content of each component of the vegetation was also determined and showed that the amounts of the major nutrients immobilised in the vegetation were: N, 1,800 lb./acre; P, 120 lb./acre; K, 800 lb./acre; Ca, 2,400 lb./acre; Mg, 350 lb./acre. The corresponding amounts of nutrients in the top foot of soil supporting the vegetation were: total N, 4,100 lb./acre; 'available' P, 11 lb./acre; exchangeable K, 580 lb./acre; exchangeable Ca, 2,300 lb./acre; exchangeable Mg, 330 lb./acre. About half the nutrients stored in the vegetation were contained in readily combustible material, and except for N would be released to the soil if the forest were cleared and burnt for cultivation. The quantity of roots and the amounts of nutrients contained in them were not sufficiently great to make an important addition to the nutrient supply in the soil during subsequent cultivation. Of the total quantity of roots supporting the vegetation 85.5 per cent by weight were within 1 foot of the soil surface.

ACKNOWLEDGEMENTS

The authors are indebted to Mr. P. H. Nye who suggested this project and collaborated in the planning of it, and to Mr. S. Boateng, Senior Soils Assistant at the Agricultural Research Station, Kade, who supervised much of the field work involved. The Chief Conservator of Forests, Ghana, Mr. A. E. Foggie, gave much helpful advice and arranged for Mr. A. A. Enti of the Forestry Department to carry out the species identification on the site.

Received May 6, 1959

REFERENCES

1 Alway, F. J., Maki, T. E. and Methley, W. J., Composition of the leaves of some forest trees. Am. Soil Survey Assoc. Bull. **15**, 81–84 (1957).

2 Bartholomew, W. V., J. Meyer and H. Laudelout, Mineral nutrient immobilisation under forest and grass fallow in the Yangambi (Belgian Congo) region, with some preliminary results on the decomposition of plant material on the forest floor. Publ. Inst. Nat. Étude Agron. Congo Belge Sér. Sci., **No. 57**, pp. 27 (1953).

3 Beirneart, A., La technique culturale sous l'équateur. Publ. Inst. Nat. Étude Agron., Congo Belge Sér. Tech. **No. 26**, pp. 86 (1941).

4 Brammer, H., The soils of Ghana, *in*: Agriculture and Land Use in Ghana. Oxford University Press (in press).
5 Dawkins, H. C., The volume increment of natural tropical high forest, and limitations on its improvement. Empire Forestry Rev. **38**, 175–180 (1959).
6 Hutton, R. G. and Nye, P. H., The rapid determination of the major nutrient elements in plants. J. Sci. Food Agr. **9**, 7–14 (1958).
7 Istas, J. R. and Raekelboom, E. L., Contribution a l'étude chimique des bois de Mayumbe. Publ. Inst. Nat. Étude Agron. Congo Belge, Sér. Sci. **No. 53**, pp. 122 (1952).
8 Nye, P. H., The relative importance of fallows and soils in storing plant nutrients in Ghana. J. W. African Sci. Assoc. **4**, 31–50 (1958).
9 Reed, W. E., Reconnaissance soil survey of Liberia. U.S. Dept. Agr. Inform. Bull. **No. 66** (1951).
10 Rennie, P. J., Uptake of nutrients by mature forest growth. Plant and Soil **7**, 49–95 (1956).
11 Taylor, C. J., The vegetation zones of the Gold Coast. Gold Coast Forestry Dept. Bull. **No. 4**, pp. 11 (1952).

APPENDIX 1

Distribution of species on measured area by girth sizes					
Species	3″–1′	1′–2′	2′–3′	> 3′	Total
Diospyros xanthoclamys	314	21	1	—	336
Strombosia glaucescens var. lucida	307	13	—	—	320
Diospyros mannii	257	5	—	—	262
Aulacocalyx jasminiflora	186	13	1	—	200
Pycnocoma macrophylla	149	—	—	—	149
Macrolobium macrophyllum	115	1	—	—	116
Baphia nitida	85	3	—	—	88
Drypetes afzelii	83	1	—	—	84
Strychnos sp.	81	1	—	—	82
Napoleona parviflora	78	1	—	—	79
Craterispermum cerinanthum	52	6	2	—	60
Microdesmis puberula	59	1	—	—	60
Rinorea oblongifolia	53	2	1	—	56
Deinbollia grandifolia	52	—	—	—	52
Marantochloa leucantha	52	—	—	—	52
Nesogordonia papaverifera	35	7	2	—	44
Polyalthia oliveri	40	1	—	—	41
Aidia genipiflora	32	2	1	—	35
Celtis mildbraedii	18	9	3	5	35
Trichilia prieuriana	21	8	6	—	35
Memecylon lateriflorum	27	4	3	—	34
Octoknema borealis	27	5	—	—	32
Blighia welwitschii	26	1	1	—	28
Xylopia quintasii	20	8	—	—	28
Maba lancea	26	—	—	—	26
Massularia acuminata	24	—	—	—	24
Glyphaea brevis	22	—	—	—	22
Heisteria parvifolia	18	3	—	—	21
Hexalobus crispiflorus	13	4	2	2	21
Scottellia coriacea	20	1	—	—	21
Cleidion gabonicum	19	1	—	—	20
Pavetta corymbosa	20	—	—	—	20
Guarea cedrata	14	1	—	1	16
Antiaris africana	13	—	1	—	14
Brieya fasciculata	14	—	—	—	14
Enantia chlorantha	10	4	—	—	14
Entandophragma angolense	8	4	2	—	14

Appendix 1 *(contd.)*

Species	3″–1′	1′–2′	2′–3′	>3′	Total
Monodora tenuifolia	14	—	—	—	14
Pachypodanthium staudtii	13	—	—	1	14
Picralima nitida	13	1	—	—	14
Sterculia rhinopetala	8	5	1		14
Amphimas pterocarpoides	12	1	—	—	13
Myrianthus arboreus	9	1	1	2	13
Piptadeniastrum africanum	10	2	—	1	13
Trichilia heudelotii	6	4	1	1	12
Uvariastrum pierreanum	10	2	—	—	12
Vitex grandifolia	11	1	—	—	12
Xylopia villosa	9	3	—	—	12
Discoglypremna caloneura	7	4	—	—	11
Maesobotrya barteri var. sparsiflora	11	—	—	—	11
Rothmannia longiflora	11	—	—	—	11
Uvariodendron giganteum	11	—	—	—	11
Others (99 spp.)	183	48	17	21	269
Total species 149 Totals	2,728	203	46	34	3,011

APPENDIX 2

Profile description of soil profiles on lower and upper parts of measured area

Depth in.	pH	Munsell colour	Description
Upper part of slope			
Surface	—	—	Thin litter layer (c. ¼ in. thick)
0–3	5.4	7.5YR 3/2	Dark brown loose silty clay loam, with many soft crumbs and very plentiful roots.
3–9	4.8	5YR 4/6	Yellowish-red firm clay loam with no obvious structural development & few roots. Many quartzite fragments and occasional ironstone concretions.
9–27	4.6	5YR 5/4	Reddish brown firm, compact clay loam with no obvious structural development. Frequent quartz gravel and ironstone concretions.
27+	5.0	7.5YR 6/6	Reddish-yellow firm compact clay loam, with no obvious structural development. Occasional quartz gravel & ironstone concretions, becoming less frequent. Traces of decomposed phyllites appear at 48 in.
Lower part of slope			
Surface		—	Thin litter layer (c. ¼ in. thick)
0–2		10YR 3/3	Dark brown loose, silty clay loam with many soft crumbs and very plentiful roots.
2–9		10YR 5/6	Yellowish-brown slightly firm clay loam, with no obvious structural development. Occasional rootlets, rare fine quartz gravel.
9–36		7.5YR 6/6	Reddish-yellow firm silty clay, with no obvious structural development. Frequent quartz gravel & ironstone concretions, becoming very frequent.
36+		7.5YR 7/6	Reddish-yellow firm clay loam, mottled yellow, with no obvious structural development. Occasional quartz gravel and ironstone concretions and pieces of decomposing phyllite.

Plant and Soil XII, no. 2 March 1960

STUDIES ON THE DECOMPOSITION OF AMINO ACIDS IN SOILS

III. THE PROCESS OF AMINO ACID AEROBIC DECOMPOSITION AND SOME PROPERTIES OF AMINO ACID-OXIDIZING ORGANISMS

by D. J. GREENWOOD* and H. LEES**

Departments of Biological Chemistry and of Soil Science, University of Aberdeen

INTRODUCTION

It has been previously shown [7 8] that most amino acids, when percolated through soil, rapidly decompose liberating large quantities of carbon dioxide and ammonia. Nevertheless, not all the added amino-acid nitrogen or amino-acid carbon is released – some is 'immobilized' and apparently then becomes more resistant to further breakdown. The object of the work was to find out the nature and properties of this 'immobilized' material which hitherto has been little studied. The paper describes percolation studies on the process of amino acid aerobic decomposition and Warburg manometric studies on soils that have just decomposed specific naturally occuring amino acids and therefore contain considerable quantities of 'immobilized' material.

MATERIALS AND METHODS

Soils.

The soils used were: – (a) The garden soil used for some of our earlier work [7 8]; this was a sandy loam, 0–25 cm, C = 3.7% [19], N = 0.4% (by

Present address:
* Department of Chemistry,
The National Vegetable Research Station,
Wellesbourne, Warwickshire. U.K.
** Department of Chemistry
Ontario Agricultural College,
Guelph,
Ontario, Canada.

Kjeldahl) and pH = 6.5 at a soil: water ratio of 1 : 2 (*w/v*). (b) Horizons of a humus iron podzol with discontinuous iron pan, Dalliefour Wood, Ballater, Aberdeenshire: A_0, 0 – 8.5 cm, C = 36%, N = 1.5%, pH = 3.6; A_1, 8.5 – 11.5 cm, C = 5.5%, N = 0.2%, pH = 3.9; A_2, 12.5 – 18 cm, C = 2%, N = 0.2%, pH 4.3; B_1, 18 – 33 cm, C = 12%, N = 0.4%, pH = 4.8. (The foregoing analyses were taken from Fitzpatrick [4], where a fuller description of the soils can be obtained.) Before use all soils were air dried and then sieved between the limits of 1.0 mm and of 4.0 mm.

Amino acids

All the amino acids were used in the DL-form except for lysine which was used in its L-form.

α-Amino-N, nitrate-N, and volatile fatty acid

Were estimated as in previous work [9].

Keto-acids

Were estimated by the method of Lu [14], the only modification being that 2*N* NaOH instead of *N* NaOH, was used for colour development. The purity of pyruvic acid was checked by the method of Friedeman and Haugen [5] which is based on analysis of the absorption spectrum of the 2,4-dinitrophenylhydrazone derivatives of the keto acids.

Lactic acid

Was estimated by the method of Barker and Summerson [1].

Soil-moisture content

About 1 g of soil, accurately weighed, was heated at 105°C for 24 h, in an open specimen bottle, transferred to a desiccator, allowed to cool, and re-weighed. The moisture content was calculated as percentage loss of original weight.

Soil-percolation and Warburg manometric techniques

The rocking [8] and forced-air [10] percolation techniques were used throughout. Warburg manometry was by standard proceedures; temperature = 30°C, 80 strokes/min, amplitude = 10 cm; filter paper + 40% KOH in centre well.

Stimulated-soil technique

Unless otherwise indicated, soils were stimulated to their substrates as described below for stimulation to leucine. The technique was somewhat similar to that used in some of the work comprehensively reviewed by

Quastel [15]. Air-dried soil (10 g) was percolated with 100 mg of leucine dissolved in 100 ml of water in the forced-air percolator [10]. The breakdown of amino acid was followed by measurements of CO_2-output and of α-amino-N in the percolate. When, according to the α-amino-N measurements, all free amino acid had disappeared from the percolate (and not later than 5 days after the amino acid was first added to the percolator) the lift tube of the percolator was raised well above the surface of the reservoir fluid so that water-saturated air continued to pass through the soil without transfer of reservoir fluid to the soil. By this means soils were freed from gravitational water in about 30 min. A soil in such a condition will be referred to as a leucine-stimulated soil. The soil was then pushed out of the soil tube of the percolator, well mixed, and immediately used for Warburg manometry. If the freshly extruded soil was too moist to handle conveniently, it was first allowed to dry a little. Great care was exercised in drying the soil at this stage; if soil is dried until there is no longer a thin film of water around all the soil particles *i.e.* if the soil is dried until it begins to change colour, it begins to lose microbial activity. Drying was therefore carried no further than was needed to bring the soil into a manipulable state.

Warburg manometric investigations of stimulated soils

A sample (1.0 to 1.5 g) of stimulated soil prepared as described above was accurately weighed into a Warburg flask. This was easily done merely by rolling up the soil in greaseproof paper, inserting the roll into the flask and pushing out the soil with a glass rod. By weighing the greaseproof paper before and after transfer of soil, an accurately weighed amount of soil could thus be cleanly transferred to the main chamber of the Warburg flask. Each flask contained 3 ml of water *i.e.* sufficient to cover the soil entirely. Substrate (usually 0.3 ml of 0.1 *M* solution of amino acid) was either added directly to the contents of the main chamber of the Warburg flask 20 min before the start of manometric readings, or tipped in from the side arm. Control manometric experiments were always run with stimulated soil and distilled water but without substrate.

Most of these studies involved the use of nitrogen-containing organic materials and, had nitrification taken place to an appreciable extent during Warburg manometry, the recorded O_2-uptakes would have included an O_2-uptake due to nitrification. However, control manometric experiments showed that under the conditions of soil stimulation used, no appreciable nitrifying activity was established in any of the soils: the O_2-uptakes recorded in Warburg manometry were therefore uncomplicated by nitrification.

Variation of leucine-metabolizing activity in the Warburg flask with the percentage leucine decomposition in the percolator.

Twelve forced-air percolators [10] were set up in the usual way, each one containing 10 g of garden soil and 100 ml of water; 100 mg of leucine was then added to eleven of them. The decomposition of leucine was followed by

measurements of CO_2-output and of α-amino-N in the percolate. When some 25 per cent of the theoretical amount of carbon added in the form of leucine had been evolved as CO_2, the percolate was removed from one of the leucine percolators and the soil briefly percolated with distilled water to remove all free leucine. The soil was then removed from the percolator, thoroughly mixed, and transferred in 1.0-g lots to Warburg flasks. The O_2-uptake of the soil was then measured in presence of 3 ml of 0.01 *M* DL-leucine or 3 ml of water added 20 minutes before the start of manometric readings. At suitable time intervals the soils from each of the remaining percolators were treated separately in a similar manner. All the manometric parts of the experiment were conducted on the same Warburg shaker and shaken at equal speeds. Control experiments confirmed that all the Warburg measurements were within the Warburg range *i.e.* the rate of O_2-uptake was not so great as to make diffusion of O_2 into the water phase a limiting factor.

Progressive drying of amino-acid-stimulated soils.

Soil (20 g) stimulated to some particular amino acid (X) was thoroughly mixed and spread out on a dish open to the air where it was allowed to dry slowly. Immediately after transfer, and thereafter at 75-min intervals, three 1-g samples of soil were removed from the dish. One of these was placed in an open specimen bottle for a total-moisture determination; the other two were transferred to separate Warburg flasks containing 3 ml of water, where the O_2-uptakes in the presence and absence of substrate (0.3 ml of 0.1 *M* X) were measured. It was thus possible to correlate O_2-uptake with total water content throughout the whole drying out process.

Effect of drying on the subsequent O_2-uptake of leucine-stimulated garden soil when percolated with water.

Garden soil (30 g) stimulated to leucine was thoroughly mixed. Half of this mixture was air dried as above until Warburg manometry on samples showed no metabolizing activity towards leucine over a period of 3 h, whilst the other half was kept moist. Weighed amounts of dried and of moist soil were transferred to separate rocking percolators containing 50 ml of distilled water and their O_2-uptakes were measured. To act as controls, O_2-uptake measurements were also made in a similar way with dried and moist weighed amounts of thoroughly mixed garden soil which had been percolated with distilled water for as long as the leucine stimulated soil had been percolated with leucine solution.

Distribution of leucine-oxidizing organisms between the percolate and different soils

The decomposition of leucine in a forced-air percolator containing a known amount of soil and 100 ml of $7.7. \times 10^{-3}$ *M* leucine was followed by measurements of α-amino-N in the percolate. A similar forced-air percolator

containing soil and 90 ml of water was set up to act as a control. When all the leucine had disappeared from the test percolate, the manometric investigations were started. Measurements of O_2-uptake of the soil were carried out as described earlier with 1.0-g samples of soil and 3 ml of water in the presence and absence of 0.3 ml of 0.1 *M* leucine, added as substrate from the side arm. Measurements of O_2-uptake of the percolate were made on 3-ml samples free of soil in the presence and absence of 0.3 ml of 0.1 *M* leucine, again added as substrate from the side arm.

The observed extra O_2-uptake due to the addition of substrate to soil or percolate was considered to be a measure of the number of leucine-oxidizing organisms in soil or in percolate. Control experiments showed that the O_2-uptake observed when substrate was added to the percolate was unaffected by the addition of small amounts of unstimulated soil to the contents of the Warburg flask.

Plate counts of leucine-oxidizing organisms

Moist leucine-stimulated soil (7 g) and water (14 ml) were shaken (120 cycles/min) for 30 min. After the resulting suspension had sedimented for 30 min, 8 ml of supernatant was pipetted into a boiling tube which was then shaken. Part of this supernatant after dilution with 0.75% saline was used for plate counts on media selective for the growth of leucine-oxidizing organisms. Measurements were made of the extra O_2-uptakes in 3 h due to the addition of 0.3 ml of 0.1 *M* leucine to 3 ml of the supernatant and to a suspension of 1.5 g of the stimulated soil in 3 ml of distilled water. The number of leucine-oxidizing organisms per g of soil (dry-weight basis) was calculated as $n.y/x$, where y is the extra O_2-uptake per g of soil (dry weight basis) obtained in 3 h due to the addition of 0.3 ml of 0.1 *M* leucine, x is the extra O_2-uptake per 3 ml of supernatant and n is the plate count per 3 ml of supernatant. The calculation depends on the assumption that the extra O_2-uptakes observed when leucine was added to soil or percolate were always proportional to the number of viable leucine-oxidizing organisms. This has support from control experiments which showed that: – (1) The extra O_2-uptakes obtained in 3 h due to the addition of 0.3 ml of 0.1 *M* leucine to 3 ml of water and varying weights of stimulated soil were proportional to the weight of soil provided it was less than 1.8 g (with greater quantities of soil there are complications due to the difficulties of O_2 penetration into the soil/water slurry). (2) The extra O_2-uptakes obtained in 3 h due to the addition of 0.3 ml of 0.1 *M* leucine to 3 ml of supernatant were not effected by the addition of 1.0 g of unstimulated soil.

No measurable extra O_2-uptakes were obtained in 3 h when leucine was added to samples of dried leucine-stimulated soil or to samples of unstimulated soil, but both sets of soil samples gave measurable plate counts. In these cases, for the sake of comparison with the moist leucine-stimulated soil, the determination of the number of viable leucine-oxidizing organisms per g of soil was made by using the same plate-count technique as was used for the moist, leucine-stimulated soil, and by making the perhaps rather doubtful

assumption that the leucine-oxidizing organisms in the dried or unstimulated soils distributed themselves between soil and supernatant in the same proportion as they did in the case of the moist, leucine-stimilated soil.

The medium used for the plate counts was: – Agar 20 g; $Ca(NO_3)_2$, 0.33 g; $CaSO_4$, 0.8 g; $MgSO_4.7H_2O$, 0.7 g; K_2SO_4, 0.025 g; K_2HPO_4, 0.005 g; $NaHCO_3$, 0.2 g; $FeCl_3$, trace; DL-leucine, 1 g; dissolved in 1 litre of water and brought to pH 6.5.

RESULTS

Decomposition of DL-*alanine. Effect of potassium nitrate.*

Other work [9] has shown that alanine breaks down in the garden soil under anaerobic conditions in the presence of nitrate with the concomitant reduction of the nitrate *i.e.* nitrate acts as an 'oxygen' donor. Table 1 shows that the O_2-uptake during aerobic alanine decomposition in the rocking percolator was unaffected by 0.02 *M* KNO_3 which suggested that nitrate was not here acting as an

TABLE 1

Decomposition of DL-alanine: Effect of potassium nitrate

The figures give O_2-uptakes (in ml) after percolating 10 g of sieved garden soil with 20 mg of DL-alanine dissolved in 50 ml of water and in 50 ml of 0.02 *M* KNO_3 in the rocking percolators at 20°C for the indicated periods of time.

Compounds percolated	Hours (from start of percolation)					
	28	48	65	89	115	161
DL-alanine	5.1	9.0	10.2	12.3	14.4	16.4
DL-alanine + 50 ml 0.02 *M* KNO_3 .	2.2	9.8	11.6	13.4	15.0	16.8

'oxygen' donor. The possibility that nitrate was reduced and then reoxidized to nitrate was excluded by the following results. Not more than 2 μg NO_2–N per ml were produced, under forced-air or rocking percolation conditions, when 100 mg alanine dissolved in 100 ml of 0.02 *M* KNO_3, or in 100 ml of 0.02 *M* KNO_3 containing a trace of thiourea (which inhibits ammonia oxidation [16]), or in 100 ml of 0.02 *M* KNO_3 plus 2×10^{-4} *M* $KClO_3$ (which inhibits nitrite oxidation [12]) were percolated through 10 g of the garden soil for four days; although in this period all the alanine had disappeared from the percolate. Both sets of experiments taken together showed that under aerobic conditions nitrate was not significantly reduced, and indicated that anaerobic processes which perhaps might be expected to occur if there were small anaerobic "pockets" in the soil were, in fact, negligible in the percolators.

The water-soluble by-products of amino acid decomposition in soil

Analyses of samples of percolate, taken during the decomposition of 100 mg of each of sixteen amino acids in the garden soil have shown that no new amino acids could be detected on the chromatograms, and that in the case of alanine decomposition likely intermediates did not exceed the following concentrations: – lactic acid 0.1 μe/ml, volatile fatty acid 0.4 μe/ml, pyruvic acid 0.02 μe/ml. Table 2 shows the relation between time and concentration

TABLE 2

Decomposition of DL-alanine

The decomposition of 200 mg of DL-alanine (*i.e.* 2.3 m-mole) dissolved in water (100 ml) by 10 g of garden soil in a forced air percolator. CO_2-output expressed as percentage of alanine-C initially added, and α-amino-N, expressed as a percentage of the α-amino-N initially added still remaining in the percolate.

Hours (from start of percolation	% CO_2	α-amino-N	Pyruvic acid (μe/100 ml)
6	0	100	0
12	0	98	0.2
19	11	87	1.0
24	18	42	2.0
35	35	0	0

of alanine and the concentration of pyruvic acid during the decomposition of alanine in the forced-air percolators. It will be noted that pyruvic acid concentration and the rate of disappearance of alanine from the percolate were maximal at the same time.

Table 3 shows that the O_2-uptake during leucine decomposition in the rocking percolator was not affected by exchange of the entire

TABLE 3

Decomposition of DL-leucine: Removal of the percolate

The figures give the O_2-uptake (ml) after the indicated time consequent upon the decomposition of 15 mg of DL-leucine in 10 g of garden soil in the rocking percolator with 50 ml of percolate at 20°C. (P.R.) indicates that in these experiments the entire percolate was replaced after 84 h from start of percolation with an equal volume of water containing the same amount of ammonia as the percolate just removed. (N.P.R.) indicates that no removal of percolate was made during this run.

Replacement of percolate	Hours (from start of percolation)							
	35	60	84	177	142	164	210	303
Leucine (N.P.R.)	2.6	10.5	12.8	15.1	16.2	17.0	18.2	19.3
Leucine (P.R.)	2.2	9.3	12.4	14.4	15.8	16.6	17.5	18.7

percolate, after 3.5 days percolation, for a solution of $(NH_4)_2SO_4$ containing NH_3-N equal to that removed in the percolate.

Effect of substrate concentration on the O_2-uptakes obtained in the Warburg flask.

Typical results showing the rates of O_2-uptake of 1.5 g of leucine-stimulated garden soil in the presence of varying initial leucine concentrations are shown in Fig. 1. It will be noted that the O_2-

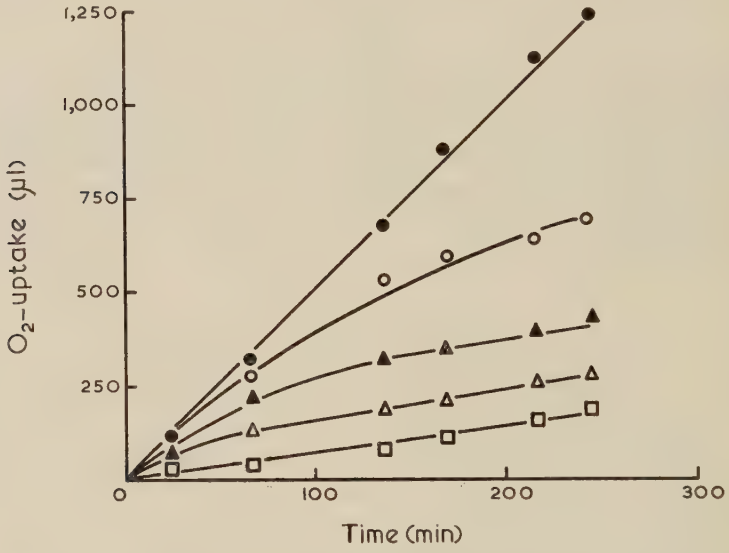


Fig. 1. Effect of substrate concentration on the O_2-uptakes obtained in the Warburg flask by DL-leucine-stimulated garden soil. Garden soil (1.5 g moist) and water (3 ml) were used and varying amounts of DL-leucine solution were added 20 min before the start of manometric readings. The number of µmole of leucine added were: – – ●– = 30 and 20 (both additions gave the same points); — ○— = 10; —▲— = 6; — △— = 3; — □— = 0.

uptakes initially follow the same straight line, but lose their linearity in order of increasing leucine concentration. A direct proportionality exists between the O_2-uptake after 4 h and the amount of leucine initially added to the Warburg flask for leucine additions of less than 10 µmole.

Variation of leucine-metabolizing activity in the Warburg flask with percentage leucine decomposition in the percolator.

Fig. 2 shows the variation in metabolizing activity as measured

in Warburg flasks of soils that in the forced-air percolator have just decomposed leucine to varying extents. The metabolizing activity was measured as the O_2-uptake resulting from the addition of leucine to the soil contained in the Warburg flask. It will be noted that the metabolizing activity reaches a peak when some 38 per

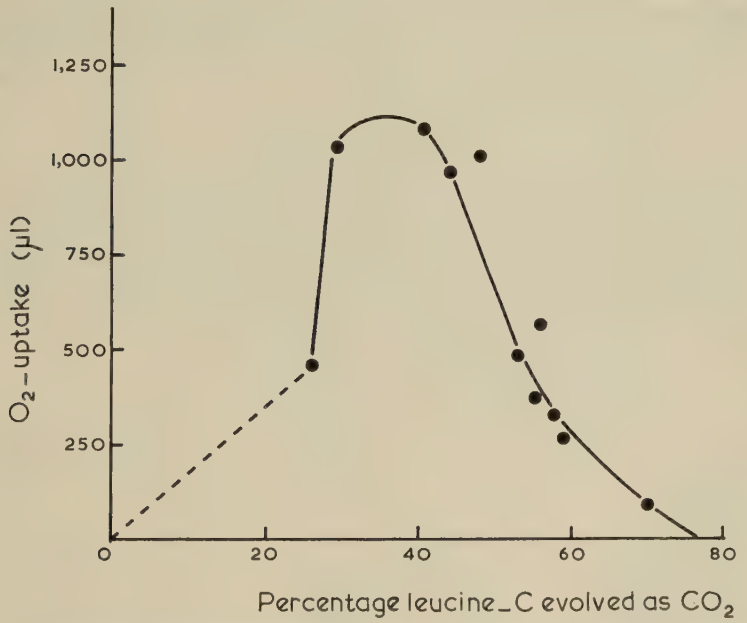


Fig. 2. Variation of leucine-metabolizing activity in the Warburg flask with the percentage leucine decomposition in the percolator. Samples of garden soil were percolated with a solution of leucine (100 mg) dissolved in 100 ml of water. Percolation was continued until measured amounts of the added leucine-C had been evolved as CO_2, when 1 g lots of these percolated soils were transferred to Warburg flasks where the respiration rates were measured in the presence of 3 ml of 0.01 *M* leucine and in the presence of 3 ml of water. *Ordinate:* Metabolizing activity *i.e.* O_2-uptake expressed in μl/4 h/g. of dry soil measured in the Warburg flask to which leucine was added minus that obtained in the flask to which no leucine was added.
Abscissa: Percentage leucine-C evolved as CO_2 in the percolator by the soil used in the Warburg flask.

cent of the percolated leucine-C has been evolved as CO_2 and the leucine has just disappeared from the percolate, and thereafter falls off sharply. The endogeneous O_2-uptakes of these leucine-stimulated soils in the absence of leucine were only 1/5 to 1/10 as great as those of the soils in the presence of leucine.

Metabolizing activities of amino acid-stimulated soils.

Garden soil was percolated in the forced-air percolators, for periods of 2 to 7 days after leucine addition. This leucine-percolated soil was then tested in the Warburg flasks with leucine and with phenylalanine as substrates. It was found that the O_2-uptake with leucine as substrate divided by the O_2-uptake with phenylalanine as substrate was approximately constant and independent of the period of percolation. Table 4 shows that for the soils studied,

TABLE 4

Metabolizing activities of soils stimulated by percolation with DL-leucine

The figures indicate the relative rates at which leucine stimulated soils can immediately metabolize different amino acids. All values calculated from the extra O_2-uptake/3 h obtained in the Warburg flask due to the addition of 30 μmol of the indicated substrate to 1 g of soil and 3 ml of water. Aspartic acid was first neutralized with NaOH. Maximum O_2-uptake due to substrate addition was in all cases 250–650 μl. Standard deviation was calculated from each set of measurements with each amino acid-stimulated soil.

Substrate	Soil		
	Garden soil	Podzol A_1	Podzol A_0
DL-alanine	86	–	98
DL-aspartic acid	83	69	102
DL-leucine	100	100	100
L-lysine HCl	25	13	33
DL-methionine	26	25	49
DL-phenylalanine	40	54	89
DL-threonine	30	32	44
No. of experiments	8	3	3
S.D.	12	11	15

despite the variations between the results obtained from experiment to experiment, there does emerge a general pattern in the relative rates at which these leucine-stimulated soils can immediately metabolize a number of other amino acids. The high standard deviation may in part be due to the high and variable values of endogenous O_2-uptakes obtained, particularly with samples from the podzol, compared with the O_2-uptakes obtained with the less readily oxidized amino acids. The relation of O_2-uptake plotted against time was more or less the same for all amino acids tested.

Warburg manometric experiments on a garden soil stimulated by previous percolation with amino acids other than leucine also revealed a pattern in the relative rates at which the stimulated soils could metabolize various substrates. This is demonstrated in Table

5. In all cases the stimulated soils showed greatest metabolizing activity in the Warburg flask towards the compound used in the percolator for stimulation. When lysine, methionine, and threonine were not used in the percolator for stimulation they were always

TABLE 5

Metabolizing activities of soils stimulated by percolation with DL-alanine, DL-aspartic acid, DL-phenylalanine, and casein

The figures indicate the relative rates at which garden soil stimulated by percolation with the indicated compounds can immediately metabolize different amino acids and casein. All values calculated from the extra O_2-uptake/3 h obtained in the Warburg flask due to the addition of 30 μmol. of the indicated substrate to 1 g of soil and 3 ml of water. Aspartic acid was first neutralized with NaOH. Maximum O_2-uptakes due to substrate addition was in all experiments between 250 and 650 μl. The average standard deviation calculated from each set of measurements was 10.

Substrate	Compound used for stimulation			
	DL-alanine	DL-aspartic acid	DL-phenylalanine	Casein
DL-alanine	100	69	69	44
DL-aspartic acid	53	100	72	–
Casein	–	–	–	100
L-leucine	25	39	34	33
L-lysine HCl	13	19	15	10
DL-methionine	14	15	22	5
DL-phenylalanine	30	35	100	29
DL-threonine	21	30	24	18
No. of experiments	5	5	3	2

TABLE 6

Metabolizing activities of soils stimulated by successive percolations of two different amino acids

Column A indicates the amino acid 70 mg of which dissolved in 100 ml of water was percolated through 10 g garden soil for 6 days. The amino acid (70 mg) under column B was then added and percolation continued until α-amino-N was no longer detectable in the percolate. Measurements were then made of the extra O_2-uptakes/3 h obtained in the Warburg flasks due to the addition of 30 μmol of DL-leucine or 30 μmol of L-lysine to 1 g of soil and 3 ml of water. The ratio of the O_2-uptake measurements due to leucine addition divided by that due to lysine addition are shown in the Ratio column.

A	B	Ratio
DL-leucine	DL-leucine	3.9
L-lysine HCl	L-lysine HCl	0.5
L-lysine HCl	DL-leucine	3.0
DL-leucine	L-lysine	0.5

metabolized in the Warburg flask more slowly than any other substrate tested.

Table 6 shows that a leucine-stimulated soil metabolized leucine more rapidly than lysine and similarly that a lysine-stimulated soil metabolized lysine more rapidly than leucine. Furthermore if a soil was first percolated with lysine and then, six days later, with leucine, the soil behaved as though it were simply leucine stimulated and *mutatis mutandis*.

Progressive drying of amino acid-stimulated soils

It will be seen from Fig. 3 that drying of a leucine-stimulated garden soil induced no loss of metabolizing activity until low moisture contents were reached. At this point the soil particles began

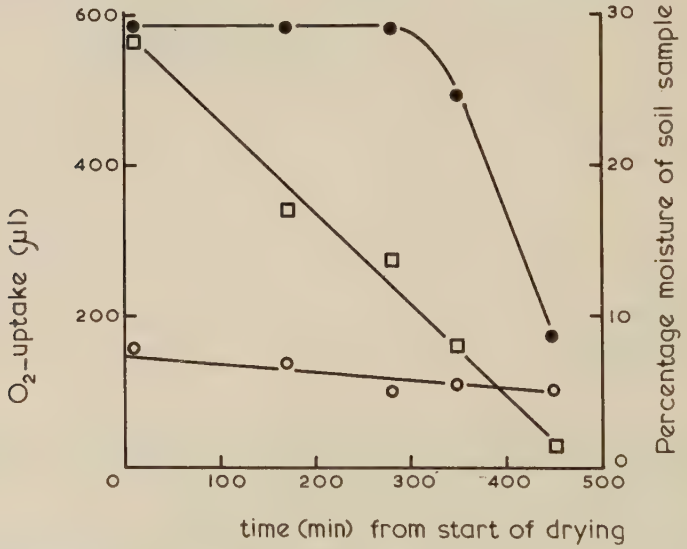


Fig. 3. Effect of progressive drying of leucine-stimulated garden soil on its metabolizing activity.
— ●— O_2-uptake, 3 h, 1.0 g of soil, 3 ml of water, 0.3 ml of 0.1 *M* DL-leucine added from side arm.
— ○— O_2-uptake, 3 h, 1.0 g of soil, 3 ml of water. No substrate addition.
— □— Percentage moisture content of soil sample.

to change colour and the soil was inactivated. Similar results were also obtained with soil samples taken from the A_1 and A_0 horizons of the podzol and with other amino acids and glucose as substrates.

TABLE 7

Effect of progressive drying of garden soil on the numbers of leucine-oxidizing organisms

Figures indicate 10^{-8} × the number of leucine-oxidizing organisms that are present in 1 g (air-dry basis) of moist and of air-dry garden soil which had been previously percolated for the same length of time with either 100 ml of water or 100 mg of DL-leucine dissolved in 100 ml of water per 10 g of garden soil until no leucine remained in the percolate. Samples of both the leucine-stimulated and unstimulated soil were air dried until (about 8 h) the leucine-stimulated soil showed no activity over 3 h in the Warburg apparatus. Average extra O_2-uptake obtained in Warburg flasks with moist samples of leucine-stimulated garden soil on addition of 0.3 ml of 0.1 *M* DL-leucine was 518 μl/g dry soil/4 h.

Time of incubation of plates (h)	72	400
Moist leucine-stimulated soil	20	24
Dried leucine-stimulated soil	0.9	1.4
Moist control unstimulated soil	0.5	0.8
Dried control unstimulated soil	0.2	0.4

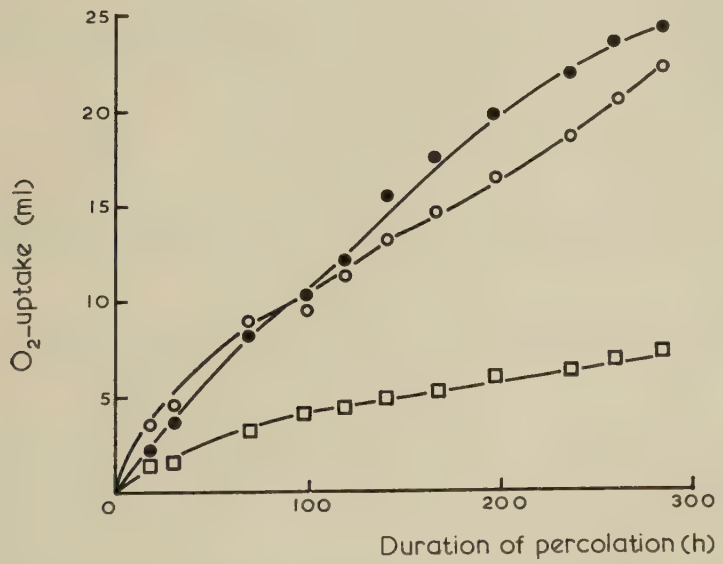


Fig. 4. Subsequent O_2-uptakes obtained by percolating 50 ml of water through 10 g (— ○ —) of dried and through 10 g (— ● —) of moist garden soil which had previously been stimulated to DL-leucine by percolation in forced-air percolators of 10 g of soil with 100 mg of DL-leucine dissolved in 100 ml of percolate. Stimulation was continued until about half of the added leucine-C had been evolved as CO_2. — □ — gives mean endogeneous O_2-uptakes of both dried and moist 10-g samples of soil which had been percolated for the same length of time with water instead of leucine solution. Soil weights are on oven-dry basis.

Metabolizing activity is here defined as the extra O_2-uptake obtained in the Warburg flasks (over a period of 3 h) due to the addition of leucine. When Warburg manometric measurements were continued for a further 8 h the extra O_2-uptake due to the addition of leucine increased exponentially. The gradient of log $_{10}$ extra O_2-uptake per time obtained from these latter measurements was about 6×10^{-5} sec^{-1}; this was a value similar to that obtained from Warburg manometric measurements on soils that had originally been stimulated with leucine but had been allowed to percolate without further leucine addition for 8 days and so showed only low leucine-metabolizing activity. Table 7 indicates that there are at least ten times as many leucine-oxidizing organisms in the moist as in the dried samples of leucine-stimulated soils, although the number of leucine-oxidizing organisms in unstimulated soil was little affected by drying.

Drying of the leucine-stimulated garden soil had no appreciable effect on its subsequent O_2-uptake during ten days subsequent percolation with water as is shown in Fig. 4. Similar results were obtained with unstimulated soil.

Distribution of leucine-oxidizing organisms between the percolate and different soils

The distribution between soil and percolate of the micro-orga-

TABLE 8

Distribution of leucine-oxidizing organisms between the percolate and different soils.

Percentage micro-organisms on soil represent the number of leucine-oxidizing organisms that were on the soil expressed as a percentage of those on the soil plus those in the percolate. Leucine-oxidizing organisms were induced by percolation of 100 mg of DL-leucine dissolved in 100 ml of water through the indicated amounts of soil. The O_2-uptakes due to the addition of 0.3 ml of 0.1 *M* DL-leucine to either 1.0 g of soil and 3 ml of water, or 3 ml of percolate were assumed, in calculating percentages, to be proportional to number of micro-organisms.

Soil	Wt. of dry soil contained in percolator (g)	Percentage micro-organisms on soil
Garden soil	10	96
A_0 horizon of podzol	4	77
A_1 horizon of podzol	6.5	31
A_2 horizon of podzol	10	50
C horizon of podzol	10	15

nisms, in a similar phase of growth, that developed in response to the addition of leucine to the soil/percolate system, are shown in Table 8. While the results are perhaps somewhat influenced by the total number of organisms present in the system and the duration of percolation, they do demonstrate that different soils apparently showed variations in the extent to which they adsorbed micro-organisms that developed in response to the addition of the same substrate.

DISCUSSION

From previous aerobic-percolation studies [8] it was not possible to say whether the amino acid metabolism being studied was proceeding by an aerobic process or a mixture of aerobic and anaerobic processes. Circumstantial evidence pointed to its being an entirely aerobic process since most amino acids decomposed with similar kinetics [8], while in the same soil under anaerobic conditions [9] some of these same amino acids were resistant to decomposition and others were not. The present work shows that although alanine decomposed in the garden soil under anaerobic conditions in the presence of excess nitrate with the concomitant reduction of nitrate [9], under forced-air or rocking percolation conditions no nitrate reduction whatever took place at any phase of the decomposition (Table 1). This seems to exclude the possibility that alanine or presumably any other amino acid, might in part, be decomposed under anaerobic conditions locally within the soil crumbs during percolation experiments.

The results here reported suggest that the decomposition of amino acids in soils under aerobic conditions is not accompanied by the extra-cellular accumulation of appreciable amounts of other amino acids or simple organic compounds. The following specific evidence may be cited: – (1) The absence of any products capable of further breakdown in the percolate as a result of continuing percolation for three and a half days after the addition of leucine to garden soil (Table 3). (2) The failure to detect in the percolates, during breakdown of amino acids, appreciable amounts of volatile fatty acids, keto acids or lactic acid (Table 2). (3) The wide range of simple organic compounds that were attacked in Warburg flasks by soils that had previously decomposed other organic compounds.

The last finding suggests that few simple organic compounds would be able to persist for appreciable periods in an actively metabolizing soil; this is in accord with the results of Bremner [2 3], Simonart and Peeters [17], and Stevenson [18]; none of whom was able to find free amino acids in appreciable amounts in aerobic soils.

Warburg manometry has shown that in practice no freshly taken sample of soil possesses enough organisms to carry out any measurable metabolism of a given amino acid within a period of four hours; *i.e.* no oxygen uptake additional to that of the soil alone was obtained within this period when the amino acid was added. If on the other hand the soil had just previously decomposed an amino acid in the percolator then it possessed a marked capacity for metabolizing that amino acid immediately when a fresh amount of it was added to the soil. The leucine-metabolizing activity of soil varied with the extent to which the leucine carbon had been evolved as carbon dioxide during percolation (Fig. 2). These experiments thus enable determinations to be made of the percentage leucine carbon not given off as carbon dioxide when the soil possessed maximum metabolizing activity in the Warburg flask. Since the metabolizing activity corresponding to particular plate counts have been determined, by making the assumption that all the added leucine not converted to carbon dioxide is converted into cellular material, the Qo_2 of the leucine-oxidizing organisms that developed in soil and the weight of each of these organisms can be calculated. From Fig. 2 it can be seen that the leucine-stimulated garden soil possessed maximum activity when 38 per cent of the leucine carbon had been given off as carbon dioxide. Therefore 62 mg of leucine per 10 g of soil was converted to leucine-oxidizing organisms. This soil had an extra oxygen uptake due to leucine addition of 280 μl/g of dry soil per h. (Fig. 2). Therefore the Qo_2 of these leucine-oxidizing organisms is $280 \div 6.2 = 46$ μl/mg/h. A similar sample of garden soil that possessed an activity of 130μl/g of dry soil per h in the Warburg flask contained 22×10^8 leucine-oxidizing organism per g of dry soil (Table 7). If this soil possessed an activity of 280 μl/g/h, it would proportionally contain $(280 \div 130) \times 22 \times 10^8$ organisms per g of dry soil. Since such a soil would contain 6.2 mg. of leucine-oxidizing organisms per g soil the weight of each organism should be about 1×10^{-12} g which is in fact the order of magnitude of a dry bacterium. Evidence for the synthesis of large quantities of

insoluble and thus possibly cellular material is given by the results of (Table 3) in which it is shown that exchange of the percolate for a solution containing the same amount of ammonia after three and a half days percolation of leucine through garden soil had no effect on its subsequent oxygen uptake rates which suggests that there was little if any decomposable organic matter in the percolate at this point.

It is notable that when leucine was percolated through a soil, the soil possessed maximum leucine-metabolizing activity when 38 per cent of the added leucine carbon had been evolved as carbon dioxide which is when the leucine just disappeared from the percolate (Fig. 2). Furthermore, only slow oxygen uptake occurs after some 36 per cent oxidation of the leucine added to a leucine-stimulated soil (Fig. 1). It appears therefore that when leucine is added to a fresh sample of soil, about 40 per cent of the leucine is first oxidized while most of the remaining leucine is synthesised into cellular material; any carbon dioxide subsequently evolved is thus probably due to the oxidation of this synthesised cellular material.

Since it has been shown by previous work [7 8 11] that large numbers of simple organic compounds decompose in several different soils with roughly the same kinetics of carbon dioxide release, it may well be therefore that in broad outline the metabolism of these compounds in most soils proceeds in roughly the same way, and thus that the general conclusions outlined above, gained as a result of studies of a few amino acids decomposing in one soil only may be of wide applicability.

Studies on the metabolizing activities of stimulated soils (Table 4) suggest that the micro-organisms that develop in soil as a result of leucine percolation possess the same general metabolizing activities, irrespective of soil type provided that the soil has a fairly high initial microbial population. Three substrates (Tables 4 and 5) were found to decompose more slowly than the remainder in stimulated soils, methionine, threonine and lysine; the anomalous behaviour of the first two has been commented on previously [8 9].

Other workers [18] have found that drying a soil reduces its endogeneous microbial population. Our work confirms this general conclusion but has shown that until the soil particles change colour drying has little effect on the metabolizing activities of the micro-

organisms, induced in soil by percolation with an organic substrate. When the soil colour begins to change, irreversible inactivation of the induced microbial population takes place (Fig. 3 and Table 7), presumably because the thin film of water surrounding the crumbs disappears. In contrast, Gleen and Quastel [6], and Quastel and Scholefield [16]have shown that neither the nitrite nor the sulphur-oxidizing organisms, induced in soil by prior percolation with the appropriate compound, are adversely affected by drying of the soil. It is notable that despite the irreversible inactivation of the immediate leucine metabolizing power on drying a leucine-stimulated soil, the rates of O_2-uptake of moist and dried samples of such a soil in the absence of leucine when remoistened are both similar and very slow (Fig. 4). It seems, therefore, that the flush in oxygen uptake that usually results on drying a soil [18], could not under our conditions of experiment account for the oxidation of more than a small proportion of the inactivated leucine-oxidizing organisms.

The studies reported on the breakdown of simple organic compounds in soil [7 8 11 20] do not seem to have revealed any clear-cut differences between their breakdown in many widely scattered soils. We have been unable to demonstrate any reproducible differences between samples from horizons of the podzol and samples of the garden soil in respect of their oxygen uptake, apart from that due to nitrification, following the addition of 15 mg of amino acid to 10 g or 6 g of soil in the rocking percolator [8]. Nevertheless the studies on the adherence of micro-organisms to soil did reveal very profound differences (Table 8). Whether the apparent adsorption of micro-organisms on soil surfaces is due to mechanical entrainment or is due to chemical bonding between soil and micro-organism, differences between soils and between different horizons of the same soil clearly do exist. It is hoped in future experiments to explore the factors governing this adsorption of micro-organisms to different soils since differences in adsorption may be of importance in elucidating soil forming processes.

SUMMARY

1. Under soil-percolation conditions the decomposition of amino acids is entirely aerobic.

2. Amino-acid decomposition proceeds under aerobic conditions without extra-cellular accumulation of appreciable concentrations of simple soluble organic compounds.

3. During leucine decomposition 40 per cent of the leucine-carbon is converted into carbon dioxide while the remaining leucine is synthesised into cellular material. Any carbon dioxide evolved thereafter is due to oxidation of this synthesised cellular material.

4. The micro-organisms induced in three different soils in response to leucine percolation had similar abilities in metabolizing different amino acids. The metabolizing abilities of micro-organisms induced in a garden soil in response to percolation with DL-alanine, or DL-aspartic acid, or DL-leucine or DL-phenylalanine have also been studied.

5. Progressive drying of soil, stimulated by percolation with specific amino acids, has little effect on its immediate metabolising activity until a low soil-moisture content is reached and the soil particles change colour. At this point the micro-organisms produced by stimulation are irreversibly inactivated.

6. The micro-organisms induced in soil/percolate systems in response to leucine percolation are adsorbed by different soils to different extents.

ACKNOWLEDGEMENTS

We wish to acknowledge our debt to Dr. W. T. H. Williamson and Professor W. O. Kermack, F.R.S., for their help, advice and encouragement throughout this work. One of us (D.J.G.) records his thanks to the Agricultural Research Council for a special research grant.

Received April 28, 1959

REFERENCES

1 Barker, S. B. and Summerson, W. H., The colorimetric determination of lactic acid in biological material. J. Biol. Chem. **138**, 535–554 (1941).
2 Bremner, J. M., Amino acids in soil. Nature (London) **165**, 367 (1950).
3 Bremner, J. M., The amino acid composition of the protein material in soil. Biochem. J. **47**, 538–542 (1950).
4 Fitzpatrick, E. A., A study of the effect of the nature of parent material on the development of the soil profile with special reference to an area in north east Scotland. Ph. D. Thesis, Univ. of Aberdeen (1951).
5 Friedemann, T. E. and Haugen, G. E., Pyruvic acid. (1) The determination of keto acids in blood and urine. J. Biol. Chem. **147**, 415–442 (1943).
6 Gleen, H. and Quastel, J. H., Sulfur metabolism in soil. Appl. Microbiol. **1**, 70–77 (1953).
7 Greenwood, D. J., Studies on breakdown of amino acids and other organic compounds in soil. Ph. D. Thesis, Univ. of Aberdeen (1957).
8 Greenwood, D. J. and Lees, H., Studies on the decomposition of amino acids in soils. 1. A preliminary survey of techniqies. Plant and Soil **7**, 253–268 (1956).
9 Greenwood, D. J. and Lees, H., Studies on the decomposition of amino acids in soils. 2. The anaerobic metabolism. Plant and Soil **12**, 68–80, (1960).
10 Lees, H., The soil percolation technique. Plant and Soil **1**, 221–239 (1949).
11 Lees, H. and Porteous, J. W., The release of carbon dioxide from soils percolated with various organic materials. Plant and Soil **2**, 231–241, (1950).

12 Lees, H. and Quastel, J. H., Bacteriostatic effects of potassium chlorate on soil nitrification. Nature (London) **155**, 276–278 (1945).
13 Lees, H. and Quastel, J. H., Biochemistry of nitrification in soil. 1. Kinetics of, and the effects of poisons on, soil nitrification as studied by a soil perfusion technique. Biochem. J. **40**, 803–815 (1946).
14 Lu, G. D., Studies on the metabolisms of pyruvic acid in normal and Vitamin B_1 deficient states. 1. A rapid specific and sensitive method for the estimation of blood. pyruvate. Biochem. J. **33**, 249–254 (1939).
15 Quastel, J. H., Soil metabolism. Proc. Roy. Soc. (London) **143B**, 159–178 (1955).
16 Quastel, J. H. and Scholfield, P. G., Biochemistry of nitrification in soil. Bacteriol. Rev. **15**. 1–53 (1951).
17 Simmonart, P. and Peeters, F., Free amino acids in humus. Trans. Intern. Congr. Soil. Sci. 5th Congr. Léopoldville **3**, 132–135. (1954).
18 Stevenson, L. L., Microbial activity in remoistened air-dried soils. Plant and Soil **8**, 170–182 (1957).
19 Walkley, A. and Black, I. A., An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. **37**, 29–38 (1934).
20 Winsor, G. W. and Pollard, A. G., Carbon-nitrogen relationships in soil. 1. The Immobilization of nitrogen in the presence of carbon compounds. J. Sci. Food Agr. **7**, 134–141 (1956).

Plant and Soil XII, no. 2 March 1960

LETTER TO THE EDITORS

Über die Brauchbarkeit der von Ed. Hofmann ausgearbeiteten Methoden zur Bestimmung der Enzyme im Boden

Einleitung

In den letzten Jahren hat Ed. Hofmann in zahlreichen Publikationen [1] [2] (dort Hinweise auf die weiteren Veröffentlichungen) über Enzyme im Boden, über deren Herkunft, Bestimmung und Bedeutung berichtet. Soweit wir feststellen konnten, beabsichtigte Hofmann die relativ umständlichen Methoden der mikrobiologischen Bodenanalyse durch exaktere Teste enzymchemischer Natur zu ersetzen.

Es bedarf hier keiner Diskussion der enzymatischen Verhältnisse der mikrobiologischen Umsetzungen im Boden und es ist selbstverständlich dass in einem Boden mit hoher Mikroorganismenzahl Enzymreaktionen deutlicher in Erscheinung treten als in mikroorganismenarmen Böden. Dass die Bestimmung einer Anzahl von Enzymreaktionen eines Bodens als Maß seiner biologischen Aktivität gegebenenfalls aufschlussreich sein kann möchten wir nicht bezweifeln, zumal wenn eine einwandfreie Methode angewendet wird.

Zu der von Hofmann gewählten Methodik möchten wir jedoch einige grundsätzliche Bemerkungen geben, die für die Beurteilung seiner Enzymteste von ausschlaggebender Bedeutung sind.

Hofmann [2] schreibt: „Bei unseren Methoden ist Toluolzusatz vorgeschrieben. Er ist notwendig, um lebende Mikroorganismen abzutöten und die in ihnen vorhandenen Enzyme in Freiheit zu setzen. Ohne Toluolzusatz würden die Lebewesen sich vermehren, Substrat sowie Spaltprodukte zersetzen und eine exakte Bestimmung verhindern. Toluolzusatz fixiert den vorhandenen Enzymgehalt...".

In den uns vorliegenden etwa zwanzig Veröffentlichungen Hofmann's und seiner Mitarbeiter über Bodenenzyme fanden wir allerdings keine experimentelle Überprüfung der Richtigkeit der von ihm behaupteten Wirkung des Toluolzusatzes auf die Bodenmikroorganismen. Abhandlungen über partielle Sterilisation des Bodens entnahmen wir aber, dass unter den von Hofmann gewählten Bedingungen eine Abtötung der Mikroorganismen in den Bodenproben unwahrscheinlich ist. So schreiben z.B. Waksman und Starkey [3]: "A small amount of toluene is thus found to have a destructive effect upon the fungi but a decidedly stimulating effect

upon bacteria and temporary depressing effect upon protozoa. By it the soil is made a much better medium for the development of fungi".

Versuchsdurchführung

Wir haben aus diesem Grunde die Keimzahlen in Bodenproben bestimmt, die genau nach den Vorschriften von Hofmann behandelt wurden (z.B. 20 g lufttrockener Boden + 2,5 ml Toluol, nach 15 min Zusatz von 10 ml Essigsäure–Na_2HPO_4–Puffer pH 5,5, sowie 10 ml 20%ige Rohrzuckerlösung). Gleichzeitig laufende Kontrollreihen bekamen anstelle von Toluol einen entsprechenden Wasserzusatz. Als Substrate wählten wir Harnstoff (Urease), Rohrzucker (Saccharase) und Stärke (Amylase). Keimzahlen wurden nach dem Koch'-schen Plattengussverfahren auf Difco-Nutrient-Broth- Glucose-Agar (pH 6,8) direkt nach Substratzugabe bzw. nach den von Hofmann angegebenen Inkubationszeiten (je nach Substrat 24–96 std) bestimmt. Eine ausgedehntere Analyse der Keimzahlen (Selektivnährböden) schien uns für den vorliegenden Zweck nicht notwendig zu sein.

Ergebnisse

Urease-Test: Zwischen Wasser- und Toluolreihen waren weder vor noch nach der Inkubationszeit wesentliche Unterschiede in den Gesamtkeimzahlen zu erkennen. Diese waren allerdings während der Inkubation sowohl bei der Wasserreihe als auch bei der Toluolreihe etwa in gleichem Maße angestiegen (in einem Versuch etwa um den Faktor 100!!).

Saccharase-Test. Die Keimzahlen blieben sowohl mit Toluol als auch mit Wasser im Zeitraum von 24 h nahezu konstant. Als geringfügiger Unterschied wurde das Fehlen von Phycomyceten auf Toluolplatten festgestellt. Die hier im Vergleich zum Ureasetest fehlende Vermehrung der Mikroorganismen kann auf die Anwendung eines für die Bakterienentwicklung ungünstigen Puffers (hohe Acetatkonzentration, pH 5,5) zurückgeführt werden.

Amylase-Test. Die Ergebnisse entsprachen etwa denen des Saccharasetestes. Einen Teil unserer Keimzahlbestimmungen haben wir in Tabelle 1 zusammengestellt. Die Keimzahlen beziehen sich auf 1 g des lufttrockenen Bodens.

Die Keimzahlbestimmungen wurden mehrmals an verschiedenen Bodenproben mit gleichen Ergebnissen wiederholt. Zwar konnten wir unter dem Einfluss des Toluols in einigen Fällen eine Verschiebung in der Artenzusammensetzung (Kolonietypen) der sich entwickelnden Mikroorganismen feststellen, auf die Gesamtzahlen an vermehrungsfähigen Keimen hatte Toluol, wenn überhaupt, nur einen unbedeutenden Einfluss.

Diskussion

Mit Recht hat Hofmann selbst darauf hingewiesen (vgl. oben), dass bei einer durch ein Substrat ausgelösten Vermehrung der Mikroorganismen

TABELLE 1

Einfluß des Toluols auf die Gesamtkeimzahlen von zwei Bodenproben in Versuchsansätzen nach den Angaben von Ed. Hofmann.

Zeitpunkt der Beobachtung	Substrate					
	Harnstoff		Rohrzucker		Stärke	
	ohne Toluol	mit Toluol	ohne Toluol	mit Toluol	ohne Toluol	mit Toluol
Bodenprobe 1						
vor Bebrütung	$6{,}4 \cdot 10^5$	$3{,}0 \cdot 10^5$	$6{,}5 \cdot 10^5$	$5{,}2 \cdot 10^5$	$5{,}3 \cdot 10^5$	$6{,}3 \cdot 10^5$
nach Bebrütung	$404 \cdot 10^5$	$318 \cdot 10^5$	$9{,}7 \cdot 10^5$	$8{,}8 \cdot 10^5$	$23 \cdot 10^5$	$14 \cdot 10^5$
Bodenprobe 2						
vor Bebrütung	$43 \cdot 10^5$	$21 \cdot 10^5$	$54 \cdot 10^5$	$41 \cdot 10^5$	$95 \cdot 10^5$	$71 \cdot 10^5$
nach Bebrütung	$930 \cdot 10^5$	$810 \cdot 10^5$	$68 \cdot 10^5$	$61 \cdot 10^5$	$274 \cdot 10^5$	$312 \cdot 10^5$

(selektive Entwicklung!) die Enzymmethode als Ausdruck einer augenblicklichen Enzymaktivität unbrauchbar ist. Aber auch dann, wenn durch die äusseren Bedingungen lediglich die Vermehrung der Organismen blockiert wird ohne dass diese abgetötet werden, kann die enzymatische Analyse restlos verfälscht werden. So wird z.B. die aus der Rohrzuckerspaltung oder Stärkespaltung freiwerdende Glucose infolge weiterer Verarbeitung durch die Mikroorganismen ihrem Nachweis (Reduktionsprobe) entzogen.

Da der grundsätzliche Fehler der von Hofmann angegebenen Methoden in einer dem Toluol nur theoretisch zugeschriebenen, aber praktisch nicht zukommenden Wirkung zu suchen ist, muss unsere Kritik natürlich auch auf die anderen, von uns nicht geprüften Enzymteste übertragen werden. Es ergibt sich hieraus, dass die von Hofmann gegebenen Werte in der von ihm gedeuteten Weise unbrauchbar sind.

Wenn Hofmann [1] unter Hinweis auf die relativ umständlichen und mit Fehlern behafteten mikrobiologischen Untersuchungsmethoden schreibt: „Dieser Umstand ist wohl Schuld daran, dass heute immer wieder von einer Förderung oder einer Schädigung des Mikroorganismenlebens im Boden gesprochen wird, ohne dass für diese Behauptung exakte Beweise vorliegen. Vielfach hat man den Eindruck, dass solche Angaben in oberflächlicher Weise und als Schlagworte gebraucht werden", so stimmen wir ihm in dieser Hinsicht durchaus zu, finden aber noch, dass auch der von ihm angegebenen, hinsichtlich der Toluolwirkung unkritischen Methode gegenüber diese Bedenken aufrecht erhalten werden sollten.

Summary

Viable cell count determinations were carried out with soil samples treated according to the methods of Ed. Hofmann for establishing the soil enzyme content. Contrary to the opinion of Hofmann the addition of toluene to the samples did not prove capable of killing the micro-organisms or limiting their growth. Under the circumstances the enzyme content of

the sample – after the addition of substrate – may change in such a way that the method worked out by Hofmann in the present form cannot fulfill the required demands.

Die Nachprüfungen wurden während unseres Aufenthaltes an der Landwirtschaftlichen Forschungsanstalt Büntehof, Hannover, durchgeführt.

D. Claus und Kl. Mechsner
Institut für Mikrobiologie der Universität, Göttingen.

Eingegangen am 3. Juli 1959.

LITERATURANGABEN

1 Hofmann, Ed., Enzymreaktionen und ihre Bedeutung für die Bestimmung der Bodenfruchtbarkeit. Z. Pflanzenernähr. Düng. und Bodenk. **56**, 68 (1952).
2 Hofmann, Ed. und Hoffmann G., Über Herkunft, Bestimmung und Bedeutung der Enzyme im Boden. Z. Planzenernähr., Düng. u. Bodenk. **70**, 9 (1955).
3 Waksman, S. A. and Starkey, R. L., Partial sterilization of soil, microbiological activities and soil fertility. I. Soil Sci. **16**, 137 (1923).

Plant and Soil will contain original contributions, as well as letters to the editors, in English, French or German. No book reviews.

Each volume will comprise *ca* 400 pages and will consist of four issues, published at irregular intervals.

The subscription price is fl. 26.50 per volume. (In the Netherlands fl. 24.—). (fl. 1.— = *ca* $ 0.27 or £ 0.1.11)

Subscriptions should be sent to the publisher:

Martinus Nijhoff - 9 Lange Voorhout - P.O.B. 269 - The Hague (The Netherlands) or to any bookseller.

Notice to the Authors

1) Manuscripts submitted for publication and communications concerning editorial matters should be sent to the Executive Editors of *Plant and Soil*, Institute for Soil Fertility, van Hallstraat 3, Groningen, Holland.

2) Manuscripts must be written in English, French or German and must be typed double space on one side of the paper, with wide margins and preferably in duplicate.

3) Papers already published in one of the above mentioned languages or under consideration elsewhere cannot be accepted.

4) It is recommended that papers be divided into sections, each headed by a caption (viz. Introduction, Methods, Experimental Results, Discussion, Summary). Historical notes should be as brief as possible. Parts of the text may be marked for small print (description of methods and analytical procedures should always be marked for small print).

5) Each paper should be concluded by a brief and clear summary in one of the above-mentioned languages.

6) Tables should be provided with headings, and diagrams with descriptive text.

Diagrams should be drawn with black Indian ink on white paper or blue tracing paper, *with the exception of the lettering*, which should be indicated in ordinary soft pencil and which will be redrawn by the publishers in order to insure uniformity.

7) References should be numbered in alphabetic order and should contain the names and initials of the authors and the title of the paper. The titles of the journals should be abbreviated in conformity with the „List of Periodicals abstracted by Chemical Abstracts" [Chem. Abstr. **50** (1956)]; *e.g.*: Winogradsky, S., Études sur les microbes fixateurs d'azote. Ann. inst. Pasteur **40**, 455-520 (1926).

8) Authors will receive 50 reprints of their articles free of charge. Additional reprints can be purchased at a nominal price.

28 Mar 61

Netherlands Journal of Agricultural Science

Quarterly Journal Edited by:

Royal Netherlands Society for Agricultural Science

Some articles published in volume 6 (1958) and 7 (1959):

Phytophtora palmivora of cocoa and its control.

The execution of a regional development plan.

Farm systems in South-East Asia.

Influence of fertilizer treatment of grassland on the incidence of hypomagnesaemia and hypomagnesaemic tetany (grass tetany) in milking cows.

Rubbergrowing in Brazil in view of the difficulties caused by South American leaf blight (Dothi della ulei).

Tobacco growing and rural welfare.

Nematodes in relation to plant growth.

Influence of phosphate on the botanical composition and on the grade of quality of herbage.

Annual subscription price: Dutch guilders f 16.— (approximately £ 1.10.0 or $ 4.25)

The journal is issued four times a year in annual volumes of about 300 pages.

Secretary: *Ir. A. Evers*, Postbox 33, Wageningen, Holland.

VERNE GRANT

NATURAL HISTORY OF THE PHLOX FAMILY

Vol. I: **Systematic Botany**

In the present study the author treats the Phlox family similarly as a model of evolution in a group of higher plants. The family is to be viewed as a model, not in the abstract sense of the theoretical and mathematical evolutionist, but rather in the sense of the naturalist - as a complex and far-flung production of nature. Three continents, but primarily western North America, are the physical setting in this model, and five tribes, eighteen generic lines, and approximately 316 species (most of these highly complex in themselves) comprise the dynamic elements in the system.

The author begins his broad comparative study with a taxonomic survey. This is followed by chapters dealing with chromosome numbers and karyotype evolution, phylogeny and phytogeography including migrational history.

= International Scholars Forum. A series of books by American scholars. *Sciences*, Vol. I.

1959. XV and 280 pp. With 1 colourplate, 79 figures and 14 tables. roy. 8vo. Cloth Guilders 19.—

Obtainable through any bookseller and direct from the publisher

MARTINUS NIJHOFF — P.O.B. 269 — THE HAGUE/THE NETHERLANDS